OPEN ACCESS


July-December 2020
ISSN 2006-9839
DOI: 10.5897/IJFA
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Vol. 12(2), pp. 21-25, July-December 2020
DOI: 10.5897/IJFA2019.0762
Article Number: E2D89AC64260
ISSN 2006-9839

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International Journal of Fisheries and Aquaculture

*Full Length Research Paper*

# Use of different colours of vertically-suspended structure during the hatchery rearing of juvenile landlocked fall Chinook salmon (*Oncorhynchus tshawytscha,* Walbaum)

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Received 15 November, 2019; Accepted 7 July, 2020

**This study investigated the effects of coloured structures on the growth of juvenile landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum) during hatchery rearing. Structures consisted of an array of four aluminum angles painted one of four colours: Silver, red, black and green, which were vertically-suspended in circular tanks. After 25 days, mean total lengths and weights of individual salmon reared in tanks with the green arrays were significantly ($P < 0.05$) larger than those reared with silver, red or black arrays. Condition factor was not significantly different ($P > 0.05$) among the colour treatments. Final total tank biomass and gain were also not significantly different ($P > 0.05$) among the colour treatments. The results of this study indicate that structural colours could be considered to maximize juvenile Chinook salmon growth during hatchery rearing.**

**Key words:** Chinook salmon, colour, *Oncorhynchus tshawytscha*, hatchery rearing.

## INTRODUCTION

Relatively little is known about the impacts of colour on cultured fish. The colours used during hatchery rearing may affect feed intake, growth, aggression, stress response and body colouration (Volpato and Bareto, 2001; Strand et al., 2007; Qin et al., 2012; Eslamloo et al., 2015; Gaffney et al., 2016; Ghavidel et al., 2019). However, the effects of specific colours are not universal among species. For example, blue light was found to reduce stress in Nile tilapia (*Oreochromis niloticus*) (Volpato and Bareto, 2001) but increase stress in rainbow trout (*Oncorhynchus mykiss*) (Karakatsouli et al., 2007). African catfish (*Heterobrachus bidorsalis*) growth improved in black tanks (Solomon and Ezigbo, 2018), but black had no impact on the growth of river catfish (*Pangasius hypophthalmus*) (Mat et al., 2019). Color preferences in fish may also change over time (Ullmann et al., 2011).

Colour and substrate interact to influence the hatchery rearing performance of gilthead seabream (*Sparus aurata*). In particular, blue and red-brown substrate

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**Table 1.** CIELAB color space digital colour values for the tank and painted arrays. L* is the black to white component, a* is the green-red component, and b* is the blue-yellow component.

| Colour | L | a* | b* |
|---|---|---|---|
| Tank: Turquoise-Green | 50.41 | -46.19 | 1.35 |
| Array: Silver | 71.48 | -1.92 | 2.01 |
| Red | 31.89 | 49.84 | 26.27 |
| Black | 9.14 | -0.99 | 1.18 |
| Green | 26.54 | -23.39 | 12.57 |

improved fish growth and reduced aggression compared to green substrate or no substrate at all (Batzina and Karakatsouli, 2012, 2014a, b). In a study comparing blue substrate, a photo of blue substrate, and a plain control, Batzina and Karakatsouli (2014b) observed positive effects on growth from only the blue substrate; the blue colour (photo of blue substrate) was not enough to produce the improvements caused by the substrate itself. Thus, interaction between colour and substrate determines effectiveness.

Vertically-suspended structure is a relatively new form of environmental enrichment. Most studies evaluating vertically-suspended structure have used unpainted aluminum angles, unpainted aluminum rods, or light grey polyvinyl chloride electrical conduit (Kientz and Barnes, 2016; Krebs et al., 2018; White et al., 2018, 2019; Jones et al., 2019). However, strings of randomly coloured spheres were used by Kientz et al. (2018) and Crank et al. (2019), which produced dramatic improvements in weight gain and feed conversion ratio in rainbow trout in comparison to those trout reared in barren tanks. While the focus of these studies was the structures themselves, both studies suggest that colour may have helped to maximize the benefits of vertically-suspended structures. The effects of colour in combination with vertically-suspended structures on fish rearing performance are unknown. In addition, the effects of colour in general during the rearing of landlocked fall Chinook salmon (*Oncorhynchus tshawytscha*, Walbaum) are also unknown. Thus, the objective of this study is to evaluate the effects of different coloured vertically-suspended arrays on landlocked juvenile Chinook salmon growth and feed conversion.

## MATERIALS AND METHODS

### Experimental design

A 25-day experiment was conducted at McNenny State Fish Hatchery, Spearfish, South Dakota, USA, using well water at a constant temperature of 11°C [water hardness as $CaCO_3$ = 360 mg/L, alkalinity as $CaCO_3$ = 210 mg/L, pH = 7.6, total dissolved solids = 390 mg/L using standard methods described by Federation et al. (2005)]. Water temperature did not vary because it originated from 100-m deep wells. On May 3, 2019, approximately 25,000 juvenile landlocked Chinook salmon originating from feral stock from Lake Oahe, South Dakota, USA (mean (± SE) length = 105 ± 3 mm, mean (± SE) weight = 12.3 ± 0.6 g, N = 30) were divided evenly into 12 turquoise-green circular tanks (diameter = 1.8 m, height = 0.8 m, water depth = 0.6 m). Each tank initially received 25.7 kg of fish (approximately 2,084 fish). The tanks were near-fully covered by corrugated black plastic overhead covers, with only 0.1 lux of light entering the tank (Walker et al., 2016). Each tank also had an array of four aluminum angles (each angle side 2.5 cm wide, 57.15 cm long), suspended from the overhead covers as described by Krebs et al. (2018). Water flows were set to maintain dissolved oxygen levels above 7 mg/L to eliminate any potential effects of relatively low oxygen levels on fish growth (Mallya, 2007) and also to maintain circular tank hydraulic self-cleaning. Substantial turbulence was only observed near the spray bar where water entered the tank.

### Colours

The four angles of the array were all of the same colour in each tank. In addition to the unpainted aluminum (silver colour), the other colour treatments were safety red, semi-gloss black, or hunter green. Treatments were randomly assigned with each coloured-array replicated in three tanks (12 total tanks with three tanks per colour). The red array was created by painting the angles with Occupational Safety and Health Administration standard coloured spray paint (Krylon, Krylon Products Group, Cleveland, Ohio, USA). The black and green arrays were created by spray painting the angles with gloss enamel (Rust-oleum, Rust-oleum Corporation, Illinois, USA). Digital colour values were obtained using a MiniScan XE Plus spectrophotometer (HunterLab, Reston, Virginia, USA) and are listed in Table 1.

### Feeding

All fish were fed 1.0 mm BioVita (BioOregon, Longview, Washington, USA) daily using automatic feeders. Aliquots of feed were dispensed for one minute at 20-minute intervals over eight hours each day. The hatchery constant method (Buterbaugh and Willoughby, 1967) was used to determine feeding rates with a projected growth rate of 0.07 cm/day and a planned feed conversion ratio of 1.1. Any mortality were removed and counted daily.

### Data collection

At the end of the experiment, five randomly sampled individual fish from each tank were both weighed to the nearest 0.1 g and total length measured to the nearest 1.0 mm. In addition, total tank weights were obtained to the nearest 0.2 kg using an Intercomp CS200 hanging scale (Medina, Minnesota, USA). The following equations were used (Piper et al., 1982):

Total weight gain = final tank weight – initial tank weight.

Feed conversion ratio (FCR) = total feed fed to tank / total tank

**Table 2.** Mean ± SE individual fish total lengths, weights and condition factors (K[a]) for Chinook salmon reared with different coloured enrichment structures. Means in a row with different letters are significantly different (P < 0.05, N = 3).

| Colour | Silver | Red | Black | Green | P |
|---|---|---|---|---|---|
| Length (mm) | 118 ± 1 z | 117 ± 3 z | 115 ± 1 z | 126 ± 2 y | 0.034 |
| Weight (g) | 16.4 ± 0.7 z | 15.7 ± 1.1 z | 15.1 ± 0.6 z | 20.7 ± 0.7 y | 0.004 |
| K | 0.99 ± 0.02 | 0.98 ± 0.02 | 0.98 ± 0.03 | 1.05 ± 0.03 | 0.201 |

[a]K = $10^5$ x [individual weight / (body length$^3$)]

**Table 3.** Mean ± SE tank total weights, gain, food fed, and feed conversion ratios (FCR[a]) for Chinook salmon reared with different coloured enrichment structures (N = 3).

| Variable | Silver | Red | Black | Green | P |
|---|---|---|---|---|---|
| Initial weight (kg) | 25.7 | 25.7 | 25.7 | 25.7 | |
| Food Fed (kg) | 14.7 | 14.7 | 14.7 | 14.7 | |
| Final weight (kg) | 37.0 ± 0.6 | 35.8 ± 0.4 | 38.2 ± 0.2 | 37.8 ± 1.7 | 0.343 |
| Gain (kg) | 11.3 ± 1.7 | 10.1 ± 0.4 | 12.5 ± 0.2 | 12.1 ± 0.5 | 0.343 |
| FCR | 1.30 +/- 0.06 | 1.45 +/- 0.05 | 1.18 +/- 0.01 | 1.21 +/- 0.15 | 0.240 |
| Mortality (%) | 0.08 ± 0.03 | 0.85 ± 0.45 | 0.06 ± 0.04 | 0.08 ± 0.02 | 0.100 |

[a] FCR = total food fed / tank gain.

weight gain.
Condition factor (K) = $10^5$ × [weight / (body length)$^3$].
Mortality (%) = 100 × (number of fish that died in a tank / initial number of fish in a tank).

#### Statistical analysis

One-way Analysis of Variance (ANOVA) was used to analyze the data with the SPSS (version 24.0) statistical analysis program (IBM Corporation, Chicago, Illinois, USA). Because the tanks were the experimental units, not individual fish, nested ANOVA was conducted on the individual fish data. If the ANOVA indicated significant differences, Fisher's Protected Least Significant Difference procedure was used for pair-wise comparisons. Significance was pre-determined at P < 0.05.

## RESULTS

Individual fish length and weight were significantly different among the colour treatments (Table 2). Individual salmon reared with the green angles were significantly heavier and longer than those reared in any of the other colour treatments. There was no significant difference in condition factor among the treatments. Total tank ending biomass, gain, and feed conversion ratio were not significantly different among the coloured angle treatments (Table 3). Percent mortality was low and not significantly different among the treatments. However, the mortality of 1.7% in one of the tanks containing the red angles was an outlier.

## DISCUSSION

The positive impact of green vertically-suspended structures on individual salmon growth after the relatively short time period of only 25 days was somewhat surprising. However, these results are supported by Luchiari and Pirhonen (2008), who observed that rainbow trout preferred green environments over those that were white, blue, yellow, or red. They also noted that the trout reared in green environments were significantly larger after 42 days. This preference could be due to the predominance of blue visual pigment cones in juvenile Chinook salmon (Flamarique, 2005), which would align most closely with the green colour used in this study. Unfortunately, blue-coloured angles were not included in this study for comparison. Ullmann et al. (2011) also described an inherent green colour preference in barramundi (*Lates calcarifer*).

Interestingly, Luchiari and Pirhonen (2008) noted that trout preference for green was temperature dependent, with rainbow trout preferring green at water temperatures of 12°C, but shifting their preference to blue at 1°C. The green colour preference for Chinook salmon in this study was obtained using 11°C water; lower temperatures were not investigated. Colour preferences in fish may not only be influenced by temperature, but also by long term exposure to different colours (Ullmann et al., 2011). It is also possible that salmon color preferences may change seasonally and with developmental stage (Beatty, 1966; Flamarique, 2005).

In contrast to the results of this study with Chinook salmon, Karakatsouli et al. (2007, 2008) reported that red light improved rainbow trout length. The red angles in the present study were associated with significantly shorter salmon in comparison to green angles. These differing results may be due to how the colour was applied (light versus structure), or the saturation of colour in the rearing environment. The red arrays presented a relatively small area of colour, whereas a red-light source would produce a much more intense colouration.

Papoutsoglou et al., (2005) observed that rainbow trout growth was reduced during rearing in black tanks. Lighter coloured tanks have been associated with increased growth, likely because feed detection is improved due to feed-background contrast (Tamazouzt et al., 2000; Papoutsoglou et al., 2005; Karakatsouli et al., 2007, 2008; Strand et al., 2007; McLean et al., 2008; Üstündağ and Rad, 2015). Strand et al. (2007) reported that at low light intensities, feed intake was higher in lighter tanks than dark tanks, but there was no significant difference at higher light intensities. Light intensity likely does not explain the results of this study, even though the tanks were nearly completely covered. The painted surfaces of the suspended angles occupied a small area in the tank, and the high L values of the silver and red colours indicate that they were lighter than the green and black angles.

While tanks containing the green angles produced significantly larger individual salmon, the possible influence of the turquoise-green rearing tank colour on these results is unknown. Other studies examining colour during rearing have focused on tank colours, rather than suspending novel colours within an already-coloured tank (Browman and Marcotte, 1987; Papoutsoglou et al., 2005; Strand et al., 2007; Eslamloo et al., 2015; Ghavidel et al., 2019). Similarly, studies examining the colour of lighting typically use tanks that are colourless, opaque, or match the lighting colouration (Karakatsouli et al., 2007, 2008; Banan et al., 2011; Elnwishy et al., 2012; Kawamura et al., 2017). The lack of significant differences in total tank gain or feed conversion ratio in this study may be due to the relatively short duration (Weathercup and McCraken, 1999), which occurred because the fish needed to be moved to make room for additional production; the tanks of salmon were only available for 25 days. The duration of this study was shorter than the minimum study duration of 56-84 days recommended by the National Research Council (2011) for fish feeding trials. In a study by de Francesco et al. (2004) differences in rearing performance from trout fed different diets did not appear until after 84 days.

The feed conversion ratios observed in this study are typical for landlocked fall Chinook salmon from Lake Oahe (Barnes et al., 2013). The relatively high rearing densities likely affected feed conversion ratio (Piper et al., 1982; Mazur and Iwama 1993; Mazur et al., 1993; Procarione et al., 1999). Feed conversion ratios of under 1.00 with this strain of Chinook salmon fed this particular diet have only been observed using very low rearing densities (Barnes et al., 2013).

In conclusion, despite the short duration of this study, significant improvements in Chinook salmon growth were observed during rearing with green-coloured vertically-suspended structures as environmental enrichment. Longer duration trials may indicate improvements in gain or feed conversion ratio and should be conducted. Additionally, experiments involving colour and Chinook salmon rearing at different temperatures, different light intensities, or different rearing tank colours would be beneficial.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENT

The authors thank Lynn Slama for his assistance with this project.

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Vol. 12(2), pp. 26-35, July-December 2020
DOI: 10.5897/IJFA2018.0671
Article Number: 97F1B7364419
ISSN 2006-9839

http://www.academicjournals.org/IJFA

International Journal of Fisheries and Aquaculture

*Full Length Research Paper*

# Effect of stocking density on growth and survival of Nile tilapia (*Oreochromis niloticus*, Linnaeus 1758) under cage culture in Lake Albert, Uganda

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Received 22 February, 2018; Accepted 16 July, 2020

**In Uganda fish is a great source of animal dietary protein, however, natural stocks continue to decline. Therefore, aquaculture provides a viable option to bridge the increasing fish supply-demand gap. Accordingly, a study was conducted from March to August 2016 to investigate the effect of stocking density on the growth performance, and survival of Nile tilapia (*Oreochromis niloticus)* in floating net-cages to contribute to aquaculture production in Uganda. Nile tilapia fingerlings, with an average weight of 4.07 g were stocked in 8 $m^3$ cages at three different stocking densities; 200, 250, and 300 fish/$m^3$, and fed on a locally formulated commercial feed for 180 days. At the end of the experimental period, results showed that fish stocked at lower densities were heavier than those stocked at higher densities. The mean final weights of fish were; 150.79±85.71, 127.82±68.43 and 118.73±49.29 g in cages stocked with 200, 250 and 300 fish/$m^3$, respectively. The mean final weight in lower density (200 fish /$m^3$) treatments was significantly higher ($P<0.05$) than that of higher density (300 fish/$m^3$) treatments. The mean relative condition factor of fish ranged from 1.02 to 1.06, but was not significantly different ($P>0.05$) among the stocking densities. Survival rate and stocking density were negatively correlated. The best survival rate (94.19%) was obtained in low stocked cages (200 fish/$m^3$) compared to 92.98% in highly stocked cages (300 fish/$m^3$). The results of this study suggest that 200 fish/$m^3$ of a cage, is the best stocking density in terms of fish growth parameters.**

**Key words:** Aquaculture, animal protein, per capita, livelihood, yield.

## INTRODUCTION

Fisheries and aquaculture play an important role as sources of food and income, and is thus critical in addressing global human food and nutrition insecurity, and economic demands (FAO, 2016). In 2013, fish contributed about 17% of the animal protein to the global human population, while the global per capita fish consumption reached 20 kg in 2014 (FAO, 2016). Additionally, fisheries and aquaculture was a source of livelihood for 56.6 million people globally in 2014 (FAO, 2016). In Uganda, fisheries and aquaculture sector significantly contributes to economic growth. The sector contributed 3% to Gross Domestic Product (GDP) in the 2015-2016 financial year. Additionally, it directly employed over 1.2 million people, generated greater than 89 million USD in export earnings, and accounted for up to 50% of animal protein food (MAAIF, 2016a). However, the global fish production from wild fish stocks has generally stagnated, with most fisheries already fully exploited or over-exploited (FAO, 2014, 2016); yet human population is increasing, thus widening the fish demand-supply gap. In Uganda, this has been accompanied by a decline in per capita fish consumption to as low as 8 kg (MAAIF, 2017) which is below the 17 kg recommended by FAO (2012). With the fast growing human population, to about 35 million people (UBOS, 2016); this per capita fish consumption will further decline due to increase in the fish demand-supply gap. Therefore, aquaculture provides one of the plausible solutions for increasing fish production, and will subsequently lessen this demand-supply gap. Moreover, Uganda has a significant potential for aquaculture development, since it has numerous water bodies that cover up to 20% of the country's total surface area (MAAIF, 2012). It also has favourable climate, good culture species such as *Oreochromis niloticus*, and availability of raw materials for feed (NAFIRRI, 2012).

As such, the Government of Uganda through her Agriculture Sector Strategic Plan (ASSP) 2016-2020, recognised the need to promote and support aquaculture in order to boost fish production to at least 300,000 tonnes annually by 2020 (MAAIF, 2016b). Cognizant of the need to meet this projected fish production target, augmenting investment in cage fish culture is one of the viable approaches that Uganda could adopt. This is because cage fish culture is known to increase fish production (Gentry et al., 2017), and can therefore result into higher returns. It, however, requires adherence to best practices including, adopting an ideal fish stocking density, in order to maximize production efficiency. Stocking density is critical since it directly influences the economic viability of fish culture enterprises (Osofero et al., 2009; Baldwin, 2010).

Stocking density directly affects the growth rate and survival of fish, and subsequently the productivity of the fish culture operations (North et al., 2006; Osofero et al., 2009; Pouey et al., 2011; Mensah, 2013). In tilapia culture, better growth performance and survival rate are obtained in lower stocking densities (Sorphea et al., 2010). On the other hand, a positive relationship between stocking density and yield of Nile tilapia was reported by Watanabe et al. (1990), Cruz and Ridha (1991), Alemu (2003) and Gibtan et al. (2008). These studies reported higher yields with increasing stocking density. Therefore, stocking densities that result into higher fish yields, and subsequently higher economic returns from a cage culture enterprise, are ideal for catalysing cage aquaculture development. However, the inadequacy of empirical information on stocking densities that result into high growth and survival rates accompanied with higher yield continues to constrain cage fish culture productivity in Uganda. Consequently, the farmer's capacity to operate cage culture systems is low, resulting into low cage fish production (Mbowa et al., 2016, 2017). Therefore, this study investigated the effects of stocking densities on the growth performance and survival of Nile tilapia (*O. niloticus)*, reared in cage facilities, to contribute to the available cage aquaculture technical information, towards increasing cage production in Uganda.

## MATERIALS AND METHODS

### Selection of study site

The study was conducted from March to August 2016 at Butiaba fish landing site, Piida Bay (20241.72 N and 313352.59 E) on Lake Albert in Buliisa District, Uganda (Figure 1). This experimental site is located in the Lake Albert Crescent Zone (LACZ) at an altitude of 616 m above sea level, approximately 68 km from the town of Hoima. Consideration of this area for the experiment was based on the baseline site suitability survey. The site is well suited for cage culture of Nile tilapia, because it is partly sheltered with an average depth of 8 m, water flow rate of 48 cm/s, pH of 7.5, Dissolved oxygen concentration 6.88 mg/L and temperature of 27.8°C, which are all within the prescribed parameters for cage fish farming (Howerton, 2001; Queensland Water Quality Guidelines, 2009).

### Experimental design

Cages of 8 $m^3$ (2 m × 2 m × 2 m), with metallic frames and an enclosure of 10 mm mesh size Nylon butane treated netting material, with 1 mm mesh size nylon cage liners were used during this experiment. The cages were secured in blocks (three per block) and fitted with cage liners as nursery cages for the small sized fish at the time of stocking. The cages were thereafter randomly stocked with monosex male Nile tilapia fingerlings, of uniform average body weight (4.07 g). The fingerlings were stocked at densities of 200, 250, and 300 fish/$m^3$ per cage, with each stocking density having 2

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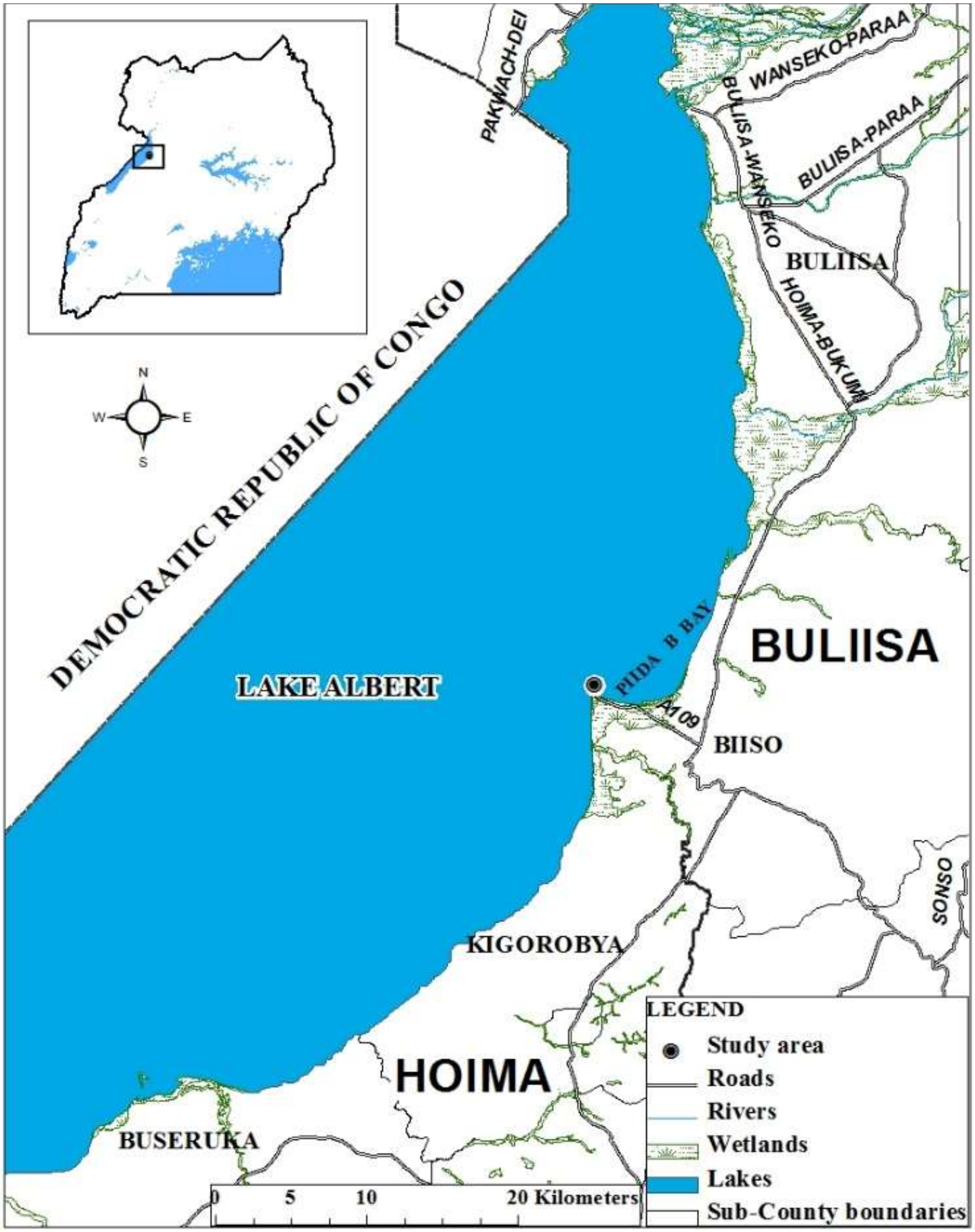


**Figure 1.** Location of Piida Bay on Lake Albert, where the experiment was conducted.

replicates. These stocking densities were based on an aquaculture policy brief, which suggested that fish stocking densities in a cage rearing facility can be high as 300 to 600 fish/$m^3$ (NAFIRRI, 2012). After two months of the experiment, the liners were removed from the cages because the fish had increased in size. Further, this was meant to abate clogging that would inhibit appropriate water exchange.

A locally produced commercial floating fish feed of 35 and 30% Crude Protein (CP) levels (based on the manufacturer's provided information) was used during the experiment. The 35% CP feed was in crumble and 2 mm pellet form, while the 30% CP feed was in a 3 mm pellet form. These different forms of the feed were adjusted based on the average body weight of the fish, and their gape (mouth) size. The 35% CP feed was initially fed to the fingerlings for a period of 3 months, while the 30% CP feeds were administered for the remaining experimental period. The fish were feed three times per day, from 09:00 am, at an interval of four hours.

### Data collection

#### *Water quality parameters*

Physico-chemical parameters (Water temperature, pH, and dissolved oxygen concentration) were measured monthly within the cages, during fish sampling in the morning hours (0900-1100 h) of the experimental period. Water temperature and Dissolved Oxygen were measured using a digital probe (YSI model, 550A), while pH was measured using a digital combo pH meter (Hanna model, H1 98129).

#### *Fish sampling*

The fish in all cages were randomly sampled at the end of every month between 08.00 – 11.00 h from March - August 2016 from cages using a scoop net (1 mm mesh size). Thirty live fish were randomly scooped out of each cage unit. Their Total length (TL) and Standard (SL) length, and individual live body weights were measured using a measuring board, and an electronic weighing scale (Model JZC-TSC-03;V5.1-2010, Minimum: 2 g, Maximum: 3000 g), respectively. The cages were also inspected and cleaned during fish sampling.

#### *Growth analysis*

Growth changes in the fish were calculated based on the following parameters; Mean Weight Gain (MWG), Condition factor, Survival Rate and Feed Conversation Ratio (FCR).

#### *Mean weights*

Monthly mean weight of fish for each stocking density was determined by averaging the individual weights of a sample of 30 individual fish in each cage. MWG was calculated as:

$MWG = Wt_2 – Wt_1$

Where, $Wt_1$ = Mean Initial weight of fish, $Wt_2$ = Mean Final weight of fish.

#### *Calculation of relative condition ($K_n$)*

A total of 1,068 *O. niloticus* individuals were used in the calculation of Relative Condition Factor (RCF). To compare fish condition among cages with different stocking densities, relative condition factor (the ratio of observed individual fish weight to expected weight of an individual of a given length), was calculated using the formula: $Kn = W/aL^b$ (Le Cren, 1951); where W is observed individual fish weight, L is observed individual fish total length, “a” is the intercept of the length-weight regression and “b” is the slope of the regression line. The values of a and b were obtained from the overall length-weight relationship ($W=aL^b$) derived by pooling data for all the cages across stocking densities. Length and weight data were log-transformed and the resulting linear regression fitted by Least Squares Method using weight as the dependent variable. A minimum of 30 individuals per stocking density was considered acceptable for computing the RCF.

#### *Feed conversion ratio (FCR)*

The amount of feed used to produce 1kg of the fish biomass was calculated as:

FCR =Weight of feed given (g)/Fish weight gain (g)

#### *Survival rate*

Daily fish mortalities were recorded during the experimental period. At the end of the culture period, all the cages were emptied and the number of fish in each cage determined. The percentage survival rate was calculated as;

Survival Rate (%) = [Number of fish at harvest / Total Number of fish stocked] × 100.

### Statistical analyses

One-way analysis of variance was used to test for differences in means of the growth performance and relative condition factor of fish, for the different stocking densities (200, 250 and 300 fish/$m^3$). Turkey’s post-hoc test was used for multiple comparisons, to study any difference among treatment means. All statistical analyses were performed with SPSS for Windows (version 20.0) at 0.05 level of significance.

## RESULTS

### Water quality

The mean values of key water quality parameters monitored throughout the study are shown in Table 1. Temperature and Dissolved Oxygen concentration ranged from 27.45±0.47 to 27.46±0.48 (°C) and 3.44±0.80 to 3.63±0.67 (mg/L) respectively, while pH ranged from 7.52±0.36 to 7.67±0.32. There were no significant differences in temperature (p=0.987), Dissolved oxygen concentration (p=0.451) and pH levels (p=0.124) across the different stocking densities in all cages.

### Fish growth performance

The mean final weight, mean weight gain, mean relative condition, percentage survival rates and FCR of fish in all the treatments at harvest are presented in Table 2. The mean weight of fish in all stocking densities increased with time during the experiment, with slight variations in the mean weights during the first four months of culture. From the fifth month, fish stocked in cages at 200 fish/$m^3$ had a higher mean weight compared to the cages with 250 and 300 fish/$m^3$ stocking densities (Figure 2). The mean final weights of fish were 150.79±85.71 g, 127.82±68.43 g and 118.73±49.29 g in stocking densities

**Table 1.** Mean values ± SD of water quality parameters in cages for 6 months at Piida Bay-Lake Albert.

| Parameter | Stocking density (fish/m$^3$) | | |
|---|---|---|---|
| | 200 | 250 | 300 |
| Temperature (°C) | 27.45±0.47 | 27.45±0.49 | 27.46±0.48 |
| Dissolved Oxygen concentration (mgl$^{-1}$) | 3.63±0.67 | 3.45±0.65 | 3.44±0.80 |
| pH | 7.55±0.344 | 7.52±0.36 | 7.67±0.32 |

**Table 2.** Growth, condition and feed conversion ratio during the experimental period.

| Parameter | Stocking densities(fish/m$^3$) | | |
|---|---|---|---|
| | 200 | 250 | 300 |
| Mean Initial weight (g) | 4.07±0.01[a] | 4.07±0.02[a] | 4.07±0.02[a] |
| Mean Final weight (g) | 150.79±85.7[a] | 127.82±68.43[ab] | 118.73±49.29[b] |
| Mean Weight gain (g) | 146.72±85.69 | 123.75±68.41 | 114.64±49.27 |
| Mean Relative condition (Kn) | 1.02±0.31[a] | 1.06±0.30[a] | 1.05±0.30[a] |
| Feed conversion ratio (FCR) | 3.16±0.01 | 3.55±1.28 | 3.68±0.07 |
| Survival rate (%) | 94.19 | 94.10 | 92.98 |

[a, b] Treatment means within the same row with different superscript letters are significantly different (P <0.05).

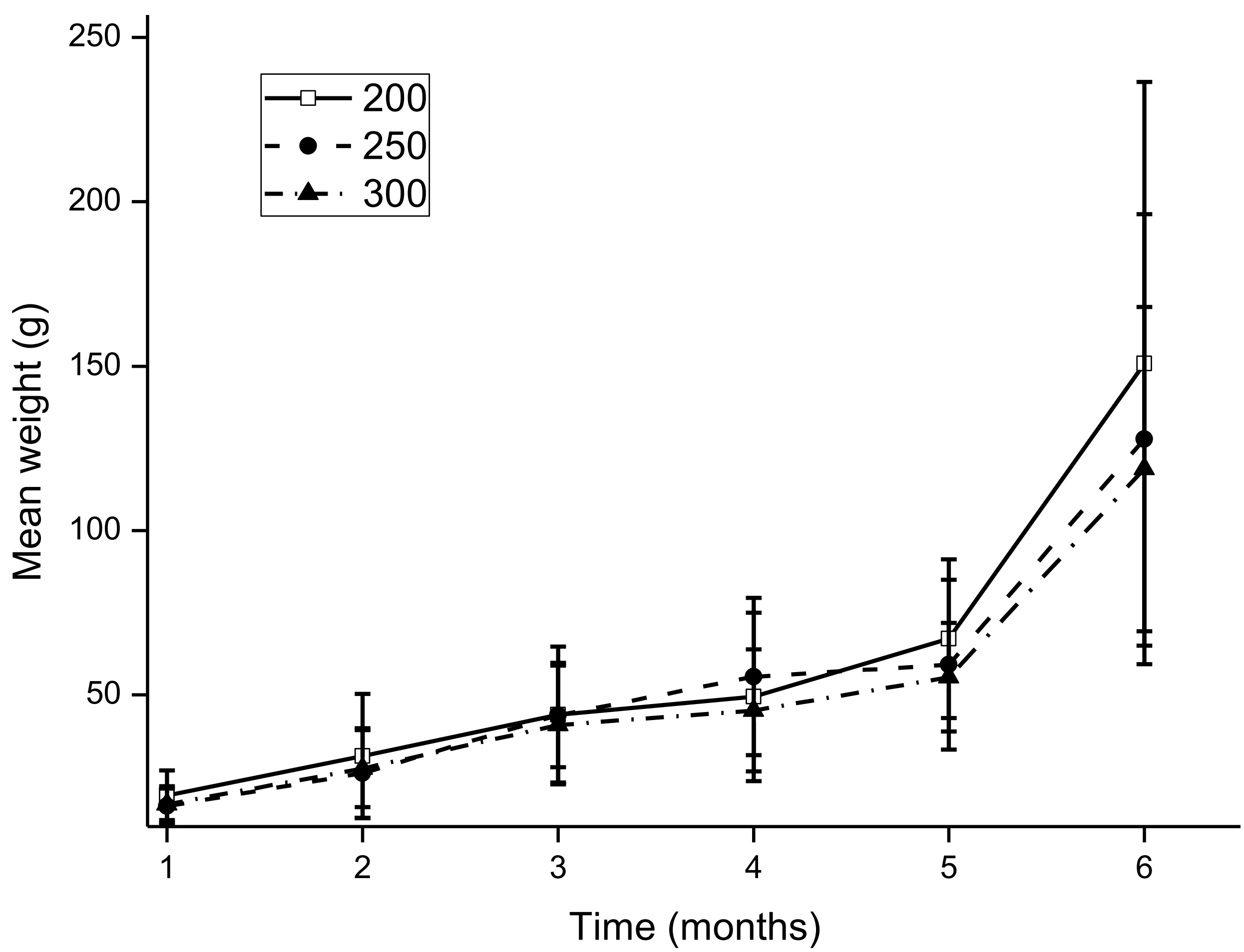


**Figure 2.** Variation in mean weight (±SD) of *O. niloticus* stocked in cages at three different densities.

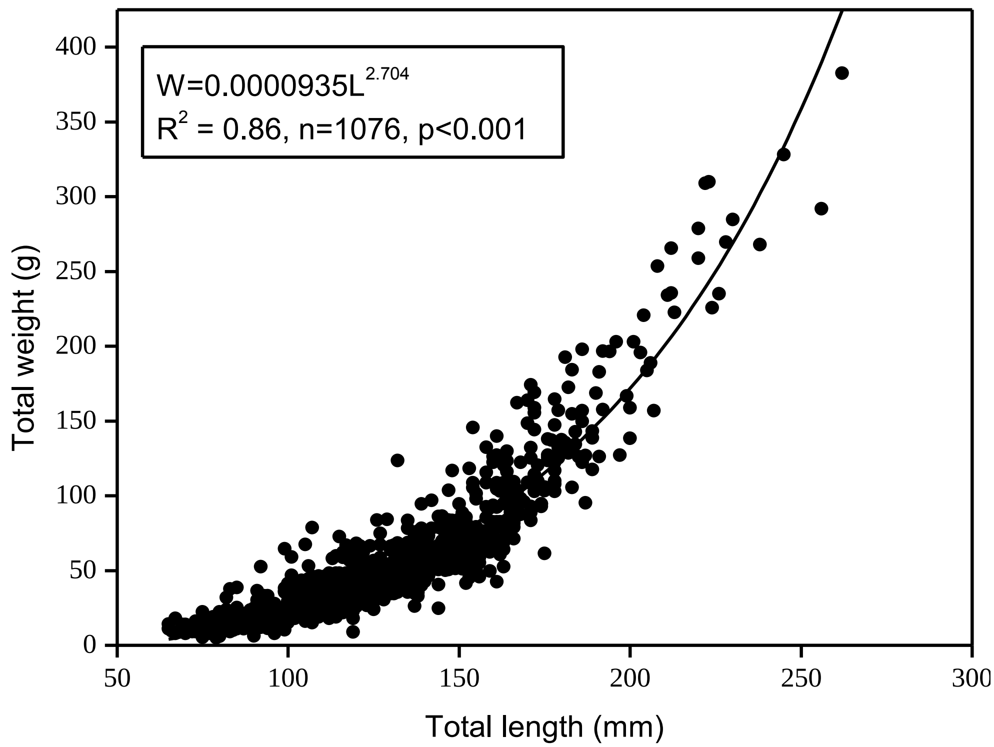


**Figure 3.** Relationships between Total length (mm) and Total weight (g) of *O. niloticus* stocked in cages at three different stocking densities.

of 200, 250 and 300 fish/m$^3$ respectively, from the initial mean weights of 4.07 g for all stocking densities (Figure 2). There was a significant difference (P<0.05) in mean final weights of the 200 and 300 fish/m$^3$ stocking densities though no significant disparity (P>0.05) was observed in mean final weights of the 200 and 250 fish/m$^3$ stocking densities.

## Length-weight relationship and relative condition factor

The size of the *O. niloticus* ranged from 120-262 mm TL and 38.2-402.2 g TW. The relationship between length and weight derived by pooling data across all stocking densities (Figure 3) was highly significant (P<0.001). Similarly, the length and weight relationship for fish in each stocking density, obtained by the regression of length and weight of individuals across treatments was significant (P<0.001). For individual stocking densities, negative allometry was obtained with 'b' values deviating from the reference b value (b=3) (Table 3). The mean relative condition of *O. niloticus* differed among stocking densities ranging from 1.02-1.06. The fish exhibited a slightly higher condition than average in the 200 fish/m$^3$ cages compared to the 250 and 300 fish/m$^3$ cages (Figure 4). However, there were no significant differences in condition of fish among the stocking densities (P>0.05).

## Survival

Overall, the survival rates across the different stocking densities had an inverse relationship with stocking density (Table 2). The survival rates (%) did not change significantly (P>0.05) amongst the three different stocking densities, and these ranged between 92.98 and 94.19. The mean survival rates (%) were 94.19, 94.10 and 92.98 for 200, 250 and 300 fish/m$^3$, respectively. The highest mean survival rate was observed in the 200 fish/m$^3$ stocking density, 250 fish/m$^3$ stocking density and lowest in the 300 fish/m$^3$ stocking density at the end of the culture period.

## Feed conversion ratio

This study did not determine the amount of feed lost during feeding, and therefore the actual amount of feed consumed by the fish. Consequently, the study compared the FCR across the three stocking densities based on the total amount of feed given during the experiment for the different treatments. The FCR ranged from 3.16 to 3.68, and increased with increase in fish stocking density. A relatively low FCR was obtained in cages stocked with 200 fish/m$^3$, followed by 250 fish/m$^3$

**Table 3.** Length-weight regressions ($W=aL^b$) for Nile tilapia from cages across the 3 stocking densities, where b=slope of regression and log10a=intercept of regression.

| Stocking density (fish/m$^3$) | n | $R^2$ | b | p |
|---|---|---|---|---|
| 200 | 356 | 0.85 | 2.71 | <0.001 |
| 250 | 355 | 0.87 | 2.66 | <0.001 |
| 300 | 357 | 0.86 | 2.77 | <0.001 |

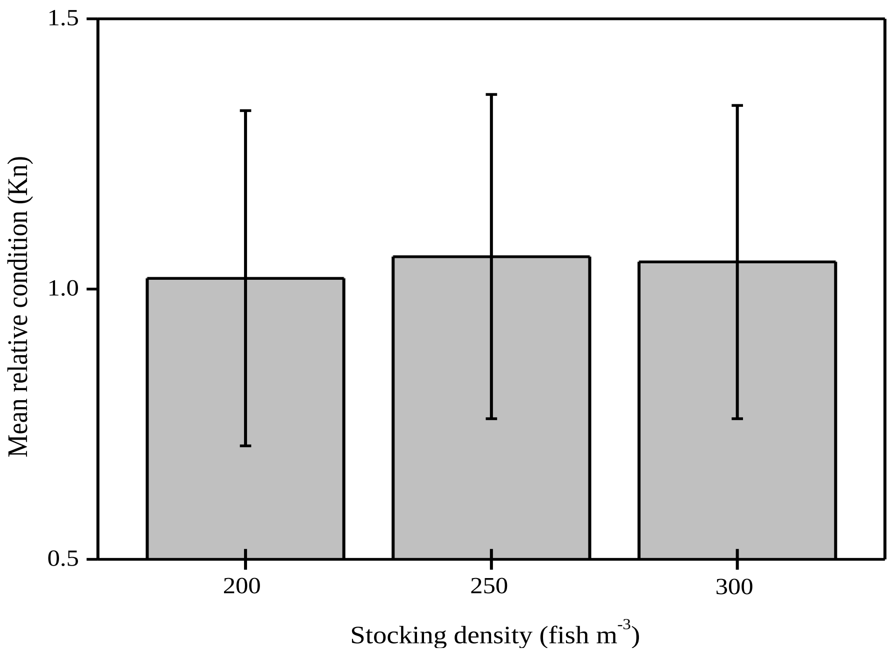


**Figure 4.** Mean relative condition factor (±SD) of *O. niloticus* stocked in cages at three different stocking densities.

and finally a higher FCR in 300 fish/m$^3$ of a cage (Table 2).

## DISCUSSION

### Water quality

Water quality greatly impacts the biology and physiology of the fish, and therefore affects the health and productivity of a fish culture system (Boyd, 2017). Consequently, maintenance of good water quality in cage aquaculture is critical for optimal growth of fish. In this study, suitable water quality parameters were maintained by regularly cleaning the cage netting to prevent fouling, which would otherwise limit appropriate water exchange within and outside the cages. Besides, the cages were installed in a site previously confirmed suitable for cage aquaculture through a site suitability study. The water parameters across all treatments ranged within accepted ranges, required optimal tilapia growth (Boyd, 2017; Popma and Masser, 2017), and thus did not have adverse effects on both growth and survival of the fish. Dissolved Oxygen (DO) concentration, pH and temperature results of this study conformed to those of Asmah et al. (2014) and Popma and Masser (2017), who recommended DO concentration and pH for Tilapia as >3 mg/L$^{-1}$ and 6-9 respectively, and the optimal temperature between 27 and 32°C as recommended by Mengistu et al. (2020). However, the low DO levels within the experimental cages could have due to the high stocking densities, since fish utilize oxygen for respiration from the waters within and around cages (Kwikiriza et al., 2016). Further, the low DO levels could be as a result of inadequate exchange of water among cages since they were clustered in blocks (Mensah et al., 2018). Overall, the obtained water quality parameter values were within the favourable ranges required for tilapia growth. Therefore, the observed differences in fish growth may not be fully attributed to the characteristics of these water quality parameters.

### Fish growth performance

The growth of Nile tilapia (*O. niloticus*) is impacted by stocking density, food quality and environmental factors such as such as Dissolved oxygen, temperature, and pH (Mensah et al., 2013; Mengistu et al., 2020). In the present study, stocking density affected the growth performance of Nile tilapia, with a negative correlation between stocking density and the growth performance of fish observed. Therefore, adopting growth performance alone, the resultant trend was that as stocking density increased, final weight gain decreased. Hence, growth performance attributed to the stocking densities showed superiority in the order 200 fish/$m^3$>250 fish/$m^3$>300 fish/$m^3$. This is in agreement with the results obtained by Asase (2013), Garcia et al. (2013) and Costa et al. (2017) that demonstrated an inverse relationship of stocking density and growth performance in tilapia cultured in net cages. Furthermore, the observed growth performance in the present study could be attributed to the differences in stocking density, since it influences fish behaviour, health, and feeding (Sanchez et al., 2010; Moniruzzaman et al., 2015; Enache et al., 2016). The observed no significant difference in the mean final weights of 200 and 250 fish/ $m^3$ stocking densities agrees with the results of a previous study by Garr et al. (2011). This study indicated that the effect of stocking density can sometimes be absent. The slow fish growth observed in the high stocked cages (300 fish/$m^3$) could be attributed to competition for limited space that resulted into the observed skin abrasions, which could have subsequently resulted into stress. This is in agreement with Roriz et al. (2017) who noted that skin abrasions and stress are observed in some fish individuals in highly stocked cages. Furthermore, M'balakaa et al. (2012) and Ronald et al. (2014) noted that although some fish species can tolerate extreme crowding, the competition for food limits their growth, resulting into poor weight gain. This was a similar scenario observed in highly stocked cages (300 fish/$m^3$), during this study. The competition for feed during fish feeding results into increased energy expenditure, and thus incurrence of greater metabolic costs. Additionally, there is increase in feed loss due to increased fish induced water turbulence in the highly stocked cages (Asase, 2013). In this study, this could have reduced the amount of food available for the fish and subsequently resulted into low growth rate in the highly stocked cages.

The length-weight relationship (LWR) is an important indicator of growth patterns and growth of fish (Silva et al., 2015). Length-weight relationships help to determine whether fish growth is isometric (b = 3) or allometric (negative allometric: b<3, or positive allometric: b>3), and also provide the condition of fish (Ricker, 1973). The fish across the stocking densities in the present study exhibited negative allometry, with the obtained "b" value lower than the reference value (b=3). This could be attributed to non-uniformity in sizes and very high variance among fish individuals. Notably, however, the values of "b" obtained in this study were in the recorded range (1.067 to 3.41) of "b" values of many fish species (Famoofo and Abdul, 2020). Similarly the "b" values in the present study were in the range of 2.299 and 3.684 recorded for Nile tilapia in the Atbara River and Khashm El-Girba reservoir, respectively (Ahmed et al., 2011). The mean Relative Condition (Kn) values of *O. niloticus* in this study were above the average condition of 1.0. This suggests that fish was in good condition (Ayode, 2011; Yosuva et al., 2018). Although Yilmaz et al. (2012) and Ali et al. (2016) noted that there may be differences in the condition factor due to sex, this was not applicable in this study since monosex male *O. niloticus* were applied in all the treatments.

## Survival

Excessive stocking density in fish culture operations has a negative influence on fish survival (Garcia et al., 2013). In Nile tilapia culture, survival rate was reported to be density-dependent (Chakraborty and Banerjee, 2012). Therefore, an ideal stocking density for Nile tilapia culture in cages must take into account of its likely impact on fish survival, since it will affect the economic returns from the enterprise. In the present study, higher survival rates (above 90%) were obtained across the treatments, though inversely proportion to stocking density. These survival rates are in contrast with the previous study by Mensah et al. (2018), which reported 70-80% survival rates in a small scale tilapia cage culture, attributed to improper fish handling and water quality deterioration. The obtained higher survival rates in the present study could be attributed to the favourable environmental conditions throughout the experimental period. Indeed, Anusuya et al. (2017) indicated that higher survival rates in Nile tilapia aquaculture could be linked to favourable physio-chemical conditions of the water body where the fish is being cultured. The high survival rates, even in cages with high stocking densities, conforms with the results of Costa et al. (2017) that indicated survival rates above 90% for stocking densities of 250, 350 and 450 fish/$m^3$ in cages. Furthermore, Sorphea et al. (2010) and Khatune-Jannat et al. (2012) noted that, high stocking densities in fish culture may at times have no effect on mortality rates, and would consequently increase fish yield. In this study however, the overall growth performance and therefore fish yield, was lowest in higher stocking densities, even when stocking density had insignificant effect on mortality.

## Feed conversion ratio

FCR expresses the ability of fish to effectively convert feed into body flesh and therefore feed use efficiency by fish. The lower values of FCR indicate that the fish has effectively converted the consumed feed into body flesh.

Therefore, the higher FCR values observed in this study, which increased with increasing stocking density, indicate poor food utilization efficiency. The FCRs in the present study deviated from the typical FCR values (1.4 to 2.5) for *O. niloticus* in African cage culture systems, as was reported by Ofori et al. (2010) and Mensah et al. (2018). However, the relatively low FCR obtained in low stocked cages (200 fish $m^{-3}$) suggests that, fish were somewhat able to extract more nutrients from the feed and subsequently converting it into flesh (Alhassan et al., 2018). The results of this study are also in agreement with Kapinga et al. (2014) and Asase et al. (2016), who indicated that feed conversion ratio increased with an increase in stocking density in tilapia culture. The observed higher values of FCR, in cages with higher fish density could be attributed to lower growth rate at higher stocking densities (Ronald et al., 2014). Additionally, this could be as a result of feed losses during fish feeding, which increases with increase in stocking density (Schmittou, 2006; Herrera, 2015). High stocking densities result in increased water turbulence during feeding, and hence increased feed losses.

## Conclusion

The effects of stocking density were evident on the growth of Nile tilapia, in terms of weight gain and subsequently the final weight of fish. The best stocking density with regard to growth performance and feed conversion efficiency was 200 fish/$m^3$ of cage. However, the resultant final weight of the fish stocked at 200 fish/$m^3$ of cage was still low. This study, therefore, recommends further research, with low stocking densities, which would result into a higher final weight of the fish, and consequently higher yields from the cages.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

The authors are grateful to the World Bank and Government of Uganda that funded this research under the Agricultural Technology and Agribusiness Advisory Services (ATAAS) project through the National Agriculture Research Organization (NARO), Uganda and appreciate the National Fisheries Resources Research Institute (NaFIRRI) and Department of Zoology, Entomology and Fisheries Sciences of Makerere University for providing technical support. We are also grateful to Buliisa District Local Government (Fisheries Department) and the participating fisher folk during the study.

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Vol. 12(2), pp. 36-46, July-December 2020
DOI: 10.5897/IJFA2020.0779
Article Number: CD8F0AC65250
ISSN 2006-9839

http://www.academicjournals.org/IJFA

International Journal of Fisheries and Aquaculture

*Full Length Research Paper*

# Socio-economic and cultural values of two species of crabs (*Cardisoma armatum* Herklots and *Callinectes amnicola* Rochebrune) in Southern Benin, Africa: Management of post-harvest losses and exoskeletons

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Received 29 May, 2020; Accepted 10 September, 2020

**Crabs play an important economic and bio-ecological role in most aquatic ecosystems and occupy an important place in the variety of trophic niches. *Callinectes amnicola* Rochebrune and *Cardisoma armatum* Herklots are two edible species widely exploited in Benin. The present work aims to i) evaluate the socio-economic and cultural importance of those two crab species, ii) analyze the dynamics of the value chain, and iii) monitor the flow of their resources. Surveys were carried out during February 2018 to September 2019 among target groups in Ouémé, Atlantic and Littoral administrative Departments of Benin through structured and semi-structured interview methods as well as free interview. Results indicate that fishermen/catchers (12.76%), wholesalers/collectors (25.53%) and retailers (61.70%) are the actors involved in the crab value chain. Between 40 and 50% of fishery products are exported to markets in Togo and Ghana. Not all of the exoskeletons resulting from post-capture losses and those resulting from treatments for consumption, in particular *C. amnicola* (74.46%) and *C. armatum* (76.59%) are used, which poses an environmental management problem. The gains made in the sector vary from 8.000±2.828 Financial Community African Franc (FCAF) (*C. amnicola*) to 20.000±21.213 FCAF (*C. armatum*). The study found that crabs (*C. armatum* and *C. amnicola*) have remarkable socio-economic importance in Southern Benin, Africa.**

**Key words:** Crab, economic analysis, monitoring, Benin.

## INTRODUCTION

In Africa, lagoon and mangrove complexes play important ecological, economic and socio-cultural roles in

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the existence of coastal communities by providing a variety of plant products, fish and crustaceans (Sowman, 2006). Among a crustaceans group, crabs take an important place. Thirty-two crab species are reported in lagoons or different habitats ranging from the marine environment to the firm ground from Côte d'Ivoire to Nigeria. These species are divided into twenty-three genera and thirteen families (Dessouassi et al., 2018). According to available literature, the highest species richness of lagoon crabs is observed in Nigeria (twenty-three species) and the lowest is observed in Togo (two species). Regional surveys conducted during colonial times and around the period of the independence movement in West Africa have not included all the lagoons in such countries as Benin or Côte d'Ivoire (Dessouassi et al., 2018). Nowadays, in lagoon systems of South Benin, the exploitation of fishery resources constitutes an essential economic activity that contributes to the livelihoods of neighboring populations (Goussanou et al., 2017a). The lagoon systems of southern Benin contain a wide variety of crustaceans, including *Cardisoma armatum* Herklots and *Callinectes amnicola* Rochebrune (Goussanou et al., 2017a; Dessouassi et al., 2018). These two abundant aquatic species are extensively caught for own consumption and trade at the Lake Nokoué (Tohozin, 2012) and Porto-Novo lagoon (Goussanou et al., 2017b).

Crab fishing in Benin aquatic environments was considered as an accessory fishery compared to the fish that made up almost all the landings. Following the fishes decrease reflected by the decrease in captures, fishermen turned to crab fishing of which exploitation intensifies from day to day in Benin aquatic environments (Goussanou et al., 2017b). Crabs are an important source of protein and several species are caught and traded by local populations (Olalekan et al., 2015). The knowledge based on lagoon crabs is variable in Gulf of Guinea countries, namely from Côte d'Ivoire to Nigeria. Some works were conducted on *C. armatum* and *C. amnicola* species in West Africa and provided information on diversity (Dessouassi et al., 2018), ecological (Shahdadi and Schubart, 2017), biology (George and Abowei, 2009), reproduction (Jivoff et al., 2007), morphological description, structural characteristics, and the growth of the two main species of crab (Goussanou et al., 2017b). Information in relation with the socio-cultural importance of these species is not available.

In general, artisanal fishermen harvest a wide variety of fish, crustaceans and molluscs and use them for various domestic purposes. Therefore, wetland organisms such as plankton, crabs and fish are linked to the human food chain. It is evident that most of the coastal areas of West Africa depend on mangroves for their survival, subsistence and income. However, several aquatic resources such as crabs are today harvested in an unsustainable and uncontrolled manner, both on a commercial scale and by artisanal fishermen (Akegbejo-Samsons and Omoniyi, 2009).

In several countries, the use of crabs is essentially the consumption of their flesh, on the other hand the exoskeletons are often regarded as waste and do not make much use. The management and impact of this waste on the environment is no less. The crustacean shells provide a high proportion of lime carbonate and a nitrogenous material, chitin, analogous to the keratin of the hairs and nails of terrestrial animals, little or no digestible, only disintegrating with extreme slowness soil where it will be buried (Gustave, 1929). The biodegradation of the shell of crustaceans is very slow (Kandra et al., 2012). There is an urgent need to treat and use crustacean waste, which contains several bioactive compounds such as chitin, pigments, amino acids and fatty acids (Kandra et al., 2012).

Literature review on the exploitation and production of *Callinectes* sp. and *Cardisoma* sp. in West Africa (d'Almeida and Fiogbé, 2008) indicates an insufficient body of knowledge about these two species despite their socio-economic relevance. The authors have suggested that knowledge be improved relative to the estimation and exploitation of stocks as well as to the biological understanding necessary for a successful breeding of *Callinectes amnicola* Rochebrune and *Cardisoma armatum* Herklots. Indeed, in Southern Benin, information on the different uses and the socio-cultural importance of *C. armatum* and *C. amnicola* is not well understood. The same applies for the use and management of the exoskeletons of the two crab species in southern Benin for better environmental management. This study aims to assess the socio-economic and cultural importance of these two crab species, and to analyze the dynamics of their value chain, as well as monitor the flow of their resources. Achieving the objectives of this work will provide new knowledge about the resources of the study area. It is expected also to improve practices and management of aquatic resources, mainly crabs, and incomes of stakeholders in the value chain of these species.

## MATERIALS AND METHODS

### Identification of the study area

The study area was identified after a documentary study that made it possible to target the large crab production areas in Southern Benin. A prospective study was carried out during February 2018 to September 2019 in these areas through meetings with community leaders and actors involved in the crab value chain. This made it possible to select areas to be taken into account for the surveys and target groups to be surveyed. Additional criteria that were taken into account included accessibility of the area and the openness of the natives to participate in the study. At the end of the prospective study, 10 municipalities and 2 districts (Figure 1) belonging to three Departments (Atlantic, Littoral and Ouémé) were selected for surveying.

### Study area

The study area belongs to the Guinean Zone between 6°25'-730'N

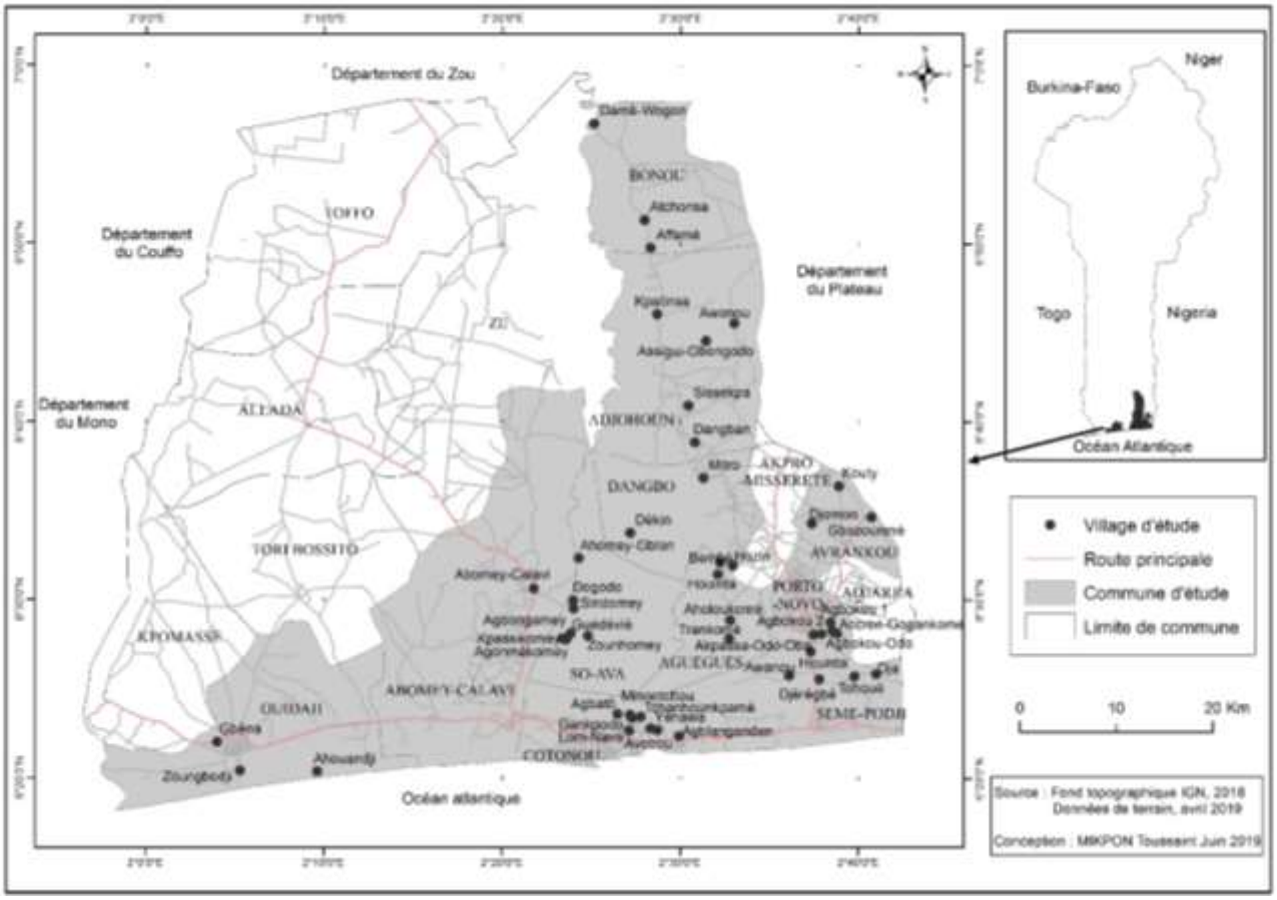



**Figure 1.** Location of the survey areas in Southern Benin, Africa.

and 2°33'-2°58'E (Figure 1) where the rainfall is bimodal (April to June and September to November) with a mean annual rainfall of 1200 mm (Assogbadjo et al., 2005). The Atlantic Department is located on a sandy coastal strip, 2 to 5 km wide and cut by lagoons and marshes that stretch along the coast. The lagoon complex of this department is increasingly salty in this case Lake Ahémé and Lake Nokoué. Lake Toho, on the other hand, contain fresh water which is mainly used for the irrigation of a 400 ha palm grove (INSAE, 2013). Average monthly temperatures range from 27 to 31°C. The months of February to April are the warmest months and the months of July to September are the coolest months (INSAE, 2013).

The Littoral Department is located on a coastal cordon whose homogeneous relief consists of a 200 km strip of alluvial sand articulated by a lagoon system. The plant cover consists of species characteristic of sandy clay and hydromorphic soils which are replaced in places by anthropogenic species. The Littoral Department does not have rivers, but Lake Nokoué (85 km²) and a few shallows constitute its water reservoirs (INSAE, 2013). The Ouémé Department belongs to the sub-equatorial region with a four season's climate. River Ouémé, Lake Nokoué and the Porto-Novo lagoon irrigate it. The vegetation has been strongly affected by various agricultural activities and now forms a mosaic of cultivated land and small relict forest patches (Assogbadjo et al., 2005). The Ouémé Department is characterized by reddish ferruginous, sandy-clay, alluvial and co-alluvial soils with vegetation which formed thicket, some relic forests, a grassland and raffia marshy formation and some mangroves (INSAE, 2013) (Figure 1).

### Description and common use of *C. armatum* and *C. amnicola*

*C. amnicola* is the swimming species with lateral and sub-lateral spines and the last pair of legs transformed into a "paddle". *C. armatum* is the walking species that has 5 pairs of legs with hair, the first pair of which is transformed into pincers. This species can be found both in water (fresh and above all brackish) and on land (swampy regions) by digging the soil to make burrows where it takes refuge. Figure 2 shows the morphology of the two species taken into account in this study. In the study area, the two crab species are used in human and animal food, medicinally and in agriculture. They are widely used (100%) in human food and are of remarkable culturally particularly in the gastronomy of the populations of the study area in the preparation of several food

### Data collection

The survey conducted during February 2018 to September 2019 used an approach based on the relationship of a population with its natural environment and the interactions therein. An organizational approach of the actors involved in the crab value chain was partially addressed. The method used for the surveys follows Dah-Nouvlessounon et al. (2015). The surveys were carried out with 3 target groups: fishermen/catchers (12), wholesalers / collectors (24) and retailers (58). Structured, semi-structured interviews as well as free interviews and observations were carried out with the


**[a] *Cardisoma armatum*** **[b] *Callinectes amnicola***

**Figure 2.** Morphology of the two crab species.

respondents using a socio-economic and cultural survey sheet designed for this purpose. The survey sheet contained 6 sections such as: Respondents' characteristics, crabs knowledge, socio-cultural importance and uses of the crabs species, dynamics and management in the value chain, crabs resource flow monitoring and socio-economic value of crabs' species. The sampling was carried out according to the method of Assogbadjo et al. (2012). It consisted of asking a question to 30 individuals in the study area. The question was whether the individual knew the two species of crab (*C. armatum* and *C. amnicola)* and was involved in the value chain. The size of the sample was determined by the formula of binomial distribution described by Dagnelie (1998):

$$n = \frac{U_{1-\alpha/2}^{2}\ x\ p(1-p)}{d^{2}} \quad (1)$$

With n the sample size; p the proportion of respondents who answered yes; d is the margin of error of the estimate; $U_{1-\alpha/2}$ is the value of the random variable at the probability value of 1-α/2. For the probability value of 0.975 (where α = 0.05), $U_{1-\alpha/2} \approx 1.96$.

#### Data analysis

Data from surveys carried out in the field were coded and entered into an Excel 2007 database. These data were analyzed with Statistical Package for the Social Sciences (SPSS) software Version 16.0 for the determination of descriptive statistics in terms of percentage and average. For socio-economic data, the GraphPad prism 7 software generated the graphs.

## RESULTS

### Socio-demographic characteristics of the actors involved in the crab value chain

A total of 94 respondents were included in the study. Indeed, the analysis of the results shows that several actors are involved in the crab value chain in the study area. These are fishermen/catchers, wholesalers/ collectors and retailers. Table 1 presents the distribution of actors according to the size of the sample and their socio-demographic characteristics relating to age, sex, level of education, religious affiliation and seniority in the field of sale and management of crabs. A rate of 54.25% of actors was enrolled in the Department of Ouémé, 19.14% in the Littoral and 26.59% in the Atlantic. Retailers (61.70%) are most involved in the chain while fishermen / catchers are the least represented in the chain (12.76%). Men (70.21%) in an age group below 30 years (60.63%) are the most represented in the value chain of the two crab species. They are mostly uneducated (54.25%) and have at least 20 years' seniority (56.38%) in the fishery resources management sector of Crabs (*C. armatum* and *C. amnicola*).

### Socio-cultural importance of the two crab species

The socio-cultural importance of the two crab species lies in the various uses that people make of them. The species *C. amnicola* is more requested (65.22%) in culinary habits (variety of dishes) than the species *C. armatum* (34.78%). In addition, according to the interviews carried out and observations made in the field, 83.33% of the respondents do not appreciate the two species to the same degree against 16.66% of the respondents who appreciate them in the same way. However, some assess according to the economic power of the species, others according to the use they make of it. The study area is made up of several ethnic and religious groups. We note a prohibition, for certain followers of the "vodoun" cult which represent 02.12% of the study population, to have contact with species *C. armatum.* This limits the relationships of this part of the population with *C. armatum*. On the other hand, *C. armatum* is used for cultural rites by other followers (05.31%), which means that this species is subject to some protection. Unlike *C. armatum*, no cultural

**Table 1.** Distribution of actors and their socio-demographic characteristics.

| Characteristics | Modality | Percentage | | |
|---|---|---|---|---|
| | | Ouémé | Littoral | Atlantique |
| Actors | Fisherman/catcher | 7.44 | 2.12 | 3.19 |
| | Wholesaler/collector | 11.70 | 5.31 | 8.51 |
| | Retailer | 35.10 | 11.70 | 14.89 |
| Sex | Woman | 14.89 | 6.38 | 8.51 |
| | Man | 39.36 | 12.76 | 18.08 |
| Age | <30 years | 34.04 | 11.70 | 14.89 |
| | Between 30 et 60 years | 7.44 | 11.70 | 39.36 |
| Education level | Uneducated | 28.72 | 11.70 | 13.82 |
| | Primary | 10.63 | 7.44 | 8.51 |
| | Secondary | 12.76 | 00 | 4.25 |
| | Higher | 2.12 | 00 | 00 |
| Seniority in the field | Between 0-20 years | 19.14 | 12.76 | 11.70 |
| | Between 21-40 years | 35.10 | 6.38 | 14.89 |
| Religion affiliation | Muslim | 2.12 | 00 | 5.31 |
| | Christian | 40.42 | 12.76 | 11.70 |
| | Animist | 10.63 | 6.38 | 9.57 |
| | Atheist | 1.06 | 00 | 00 |

**Table 2.** Reported use of *C. amnicola* and *C. armatum* crabs in the study area.

| Species | Used Part | Proportion (%) of different uses | | | | |
|---|---|---|---|---|---|---|
| | | Human food | Animal feed | Medicinal | Agricultural | No use |
| *C. amnicola* | Flesh | 100 | 8.51 | 7.44 | 00 | 00 |
| | Exoskeletons | 2.12 | 18.08 | 00 | 5.31 | 74.46 |
| *C. armatum* | Flesh | 100 | 15.95 | 3.19 | 00 | 00 |
| | Exoskeletons | 3.19 | 6.38 | 10.63 | 8.51 | 76.59 |

prohibitions and rites have been reported for *C. amnicola* apart from the reasons of allergy and preferably mentioned by those who do not consume it regularly.

Table 2 shows that different parts (the flesh and the exoskeletons) of these crabs are used in varying proportions. So in human food, it is mainly the flesh (100%) of the two species that is used. In contrast, the exoskeletons of *C. amnicola* (18.08%) and *C. armatum* (6.38%) are used as a substitute for shells in the production of animal feed. Medicinally, the powder of dried *C. armatum* exoskeletons (10.63%) is used alone or in combination with other natural products in the treatment of wounds (burn victims and accident victims). Likewise, the exoskeletons of *C. amnicola* (5.31%) and *C. armatum* (9.57%) are used in agriculture for soil fertilization. Besides these, a large part of the study population does not use and does not recognize any use of the exoskeletons of *C. amnicola* (74.46%) and *C. armatum* (76.59%)

## Dynamics and management in the value chain of *C. amnicola* and *C. armatum*

The actors involved in the value chain of the two species are fishermen/catchers, wholesalers/collectors and

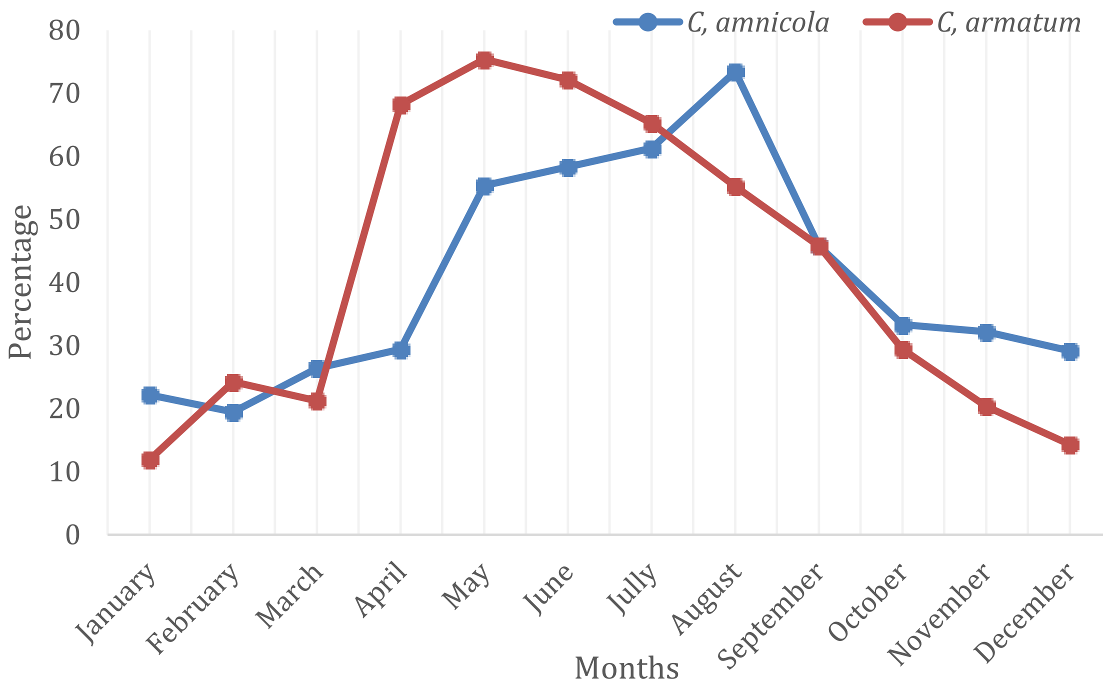



**Figure 3.** Periods of availability of the two crab species in the study area.

retailers. The fishermen/captors being the first actors in the chain use various engines in the fishing and the capture of the two species in the lagoon systems of the study area. These include wide mesh nets, the tyres and other traps made for this purpose. *C. armatum* is fished/captured in the lagoon systems of Lake Nokoué - Porto-Novo Lagoon, Ouémé valley and the mangroves. *C. amnicola* is much more fished in the lagoon system of Lake Nokoué - Porto-Novo lagoon. For the fishing/capture of *C. armatum*, the young men are the most numerous (58.33%) at this level of the chain. On the other hand, for the *C. amnicola* fishery, women are more involved (66.66%).

Products from fishing/capture are delivered to wholesalers/collectors who are at the second link in the chain. At this level of the chain, 40-50% of fishery products are exported to the markets of Togo and Ghana. The actors at this level of the chain (wholesalers/ collectors) are more or less organized and collaborate together to maintain their domination power over the other actors in the chain. Retailers are at the third level of the chain and are the most numerous (61.70%). They obtain their supplies from wholesalers/collectors and are most active at points of sale and markets in the neighboring localities of the study area. At all levels of the chain, from fishermen/captors to retailers, products are delivered fresh, which complicates post-catch management. There are many losses in post-catch management. *C. armatum* is more resistant than *C. amnicola*. However, each actor takes steps to minimize losses after capture. Both among the actors (fishermen/ captors and retailers) involved in the distribution of products (*C. armatum* and *C. amnicola*) as well as among consumers, 100% of the exoskeletons resulting from post-capture losses as well as those that are derived treatments for consumption, in particular 74.46% (*C. amnicola*) and 76.59% (*C. armatum*) are not used in any way.

## Resource flow monitoring of *C. amnicola* and *C. armatum*

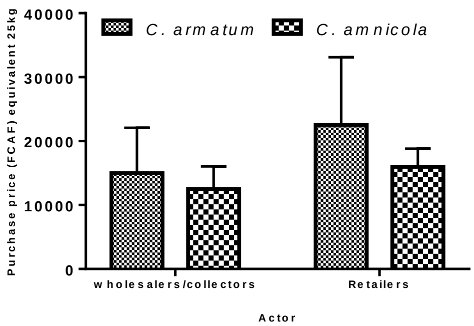
Both species are available throughout the year with a fluctuation in abundance depending on the season. Figure 3 shows the availability periods of the two species in the markets and points of sale. Analysis of the figure shows that the two species have an abundance between April and August. In addition, the availability of *C. armatum* is more noticeable (75.35%) in May while that of *C. amnicola* is more abundant (73.45%) in August. In addition, between September and March, demand is stronger than supply, which leads to soaring prices for the two species at these times of the year.

## Socio-economic value of *C. amnicola* and *C. armatum*

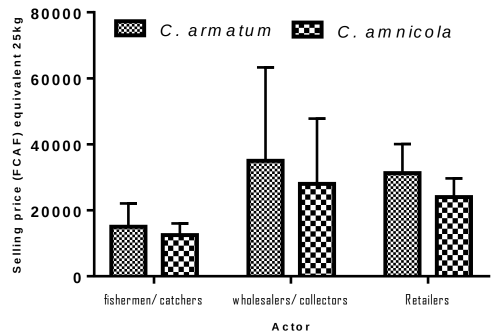
Information gathered during market surveys on the crab trade has shown that the different actors involved in the industry do not use the same sales strategies in the value chain of fishery products. Indeed, the units of

[a] Retailer of *C. amnicola* [b] Retailer of *C. armatum* [c] Fishermen and wholesalers

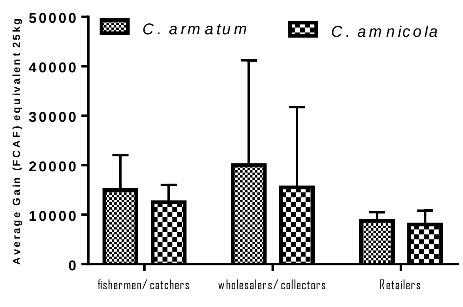
**Figure 4.** The units of measurement in the crab sales chain according to the actors.




[a] Purchase price




[b] Selling price




[c] Cost price

**Figure 5.** Variation in purchase, selling prices and average earnings depending on the actors involved in the sector.

measurement and the quantities sold vary greatly according to the species. For the two species (*C. amnicola* and *C. armatum*), fishermen/catchers as well as wholesalers/collectors use 25 kg equivalence baskets for trade (sale and purchase). However, for *C. amnicola*, retailers use smaller units of measure (basket and plastic) depending on the quantity desired by the buyer. In addition, for *C. armatum*, retailers use batches of 4 to 5 crabs tied in nets. For *C. armatum*, at the level of all actors in the chain, the quality and price of the product is linked to the size, sex and maturity of the crabs. The females crab engrained of eggs are very appreciated for consumption and are more expensive. Figure 4 shows the different units of measurement in the crab sales chain. The purchase and sale prices as well as the gains made vary depending on the actors involved in Crab value chain (Figure 5).

For *C. armatum*, the average purchase price of 25 kg equivalence varies from 15.000±7.071 FCAF (wholesalers/collectors) to 22.500 ± 10.606 FCAF (retailers). In addition, the average selling prices vary from 15.00±7.071 FCAF (fishermen / catchers) to 35.000±28.284 FCAF (wholesalers/collectors). In fact, the gains made vary from 8.750±1.767 FCAF (retailers) to 20.000±21.213 FCAF (wholesalers/collectors). Wholesalers/collectors are those who make more profit with high added value in the sector. Note that the selling price of an equivalent of *C. armatum* to local retailers is two times lower than that of buyers in the sub-region (Togo and Ghana). For *C. amnicola*, the average 25 kg equivalence purchase prices vary from 12.500±3.535 FCAF (wholesalers/collectors) to 16.000 ± 2.828 FCAF (retailers). In addition, the average selling prices are 24.000 ± 5.656 FCAF at retailers and 28.000±19.798 FCAF at wholesalers/collectors. In fact, the gains made vary from 8.000±2.828 FCAF (retailers) to 15.500±16.263 FCAF (wholesalers/collectors). Along with the species, wholesalers/collectors are also those who make more profit in the sector. Actors can sell an equivalent of 50 kg of product per day on the market during periods of abundance.

## DISCUSSION

### Socio-demographic characteristics of the actors involved in the crab value chain

The lagoon systems of Southern Benin contain a wide variety of crustaceans including *C. armatum* and *C. amnicola* (Tohozin, 2012; Hinvi et al., 2013). Through a structured survey method, the study assessed the socio-economic and cultural importance of the two species and analyzed the value chain of these species as well as monitoring the flow of their resources. Socio-demographic characteristics of respondents show that at the first level of the chain, young people (21 to 30 years old) are the most represented, constituting 60.63% of the sample. This observation was also made in Ivory Coast by Sankaré et al. (2014b) and in Madagascar by Razafindralambo (1992) during a survey on the contribution of maritime and artisanal fishing in the socio-economics life of the fishermen. This phenomenon is common because the fishing activity requires young individuals suited to the physical strength and requirement. Experienced individuals aged between 31 and 45 years normally tend to then leave this level. Besides, in sex/gender aspect, Sankaré et al. (2014a) showed that individuals aged between 20 and 45 years, the proportion of young female (33.5%) was important compared to the young male proportion (29%). The same observation was made in our study for the *C. amnicola* fishery; women are more involved (66.66%). Others studies (Sankaré, 2007; Gnimadi et al*.,* 2008) showed that women play central role in crabs' chain value.

Among the constraints to the development of small-scale fishing, Horemans (1994) pointed out that socio-cultural constraints are linked to the generally low level of education and the social status of the small-scale fisherman who in many countries receive little consideration. In addition, some of them make fishing a subsistence activity or even divide their time between fishing and agriculture.

### Socio-cultural importance of the two crab species

*C. armatum* and *C. amnicola* have a socio-cultural importance which appears through their various uses. Like other fishery products (fish and shrimp), *C. armatum* and *C. amnicola* are highly valued and constitute fundamental components of the culinary recipes of the populations of the study area and those of other countries such as Côte d'Ivoire (d'Almeida et al., 2014), Nigeria (Olalekan et al., 2015, Jerome et al*.,* 2020) and certain Latin American countries (Vasquez and Ramirez, 2015). Both species are highly valued and are sometimes substituted for other fishery products (fish, shrimp and others). This attitude of the study population is linked to the composition and nutritional value of crabs because crabs for the majority of species are a good source of protein and mineral compounds (Vigneshwari and Gokula, 2020). In many countries, crabs' meat has become a favourite food for many people replacing red meat and chicken due to the presence of high nutritive elements (Vigneshwari and Gokula, 2020). It is noteworthy that crab meat contains lower calories than beef, pork and poultry (Jimmy and Arazu, 2012). It is particularly rich in Omega-3 fatty acids, which are necessary to lower triglycerides and blood pressure, thereby reducing the risk of heart disease (Williams et al., 2016). It is also an excellent source of many vitamins (B2, B3, B12 and C) and minerals like iron, calcium, potassium, phosphorus and zinc, which aids in reducing oxidative damage to cells and tissues and acts as an antioxidant by cancelling out the carcinogenic effects (Soundarapandian et al*.,* 2014). Proximate composition of *C. amnicola* showed more carbohydrate content in crunchy chest followed by in walking legs and in tissues (Moronkola et al*.,* 2011). Medicinally, the powder of dried *C. armatum* exoskeletons (10.63%) is used alone or in combination with other natural products in the treatment of wounds (burn victims and accident victims). Other authors also report the medicinal use of crabs. Dobson (2004) reports that crabs are put to various medicinal uses. One of the most interesting is the role of *Potamonautes raybouldi*, the tree hole crab of the East Usambara Mountains in Tanzania and the Shimba Hills in Kenya (Bayliss, 2002, Cumberlidge and Wranik, 2002). Here it is not the crab itself that is important, but the water from the tree hole in which it lives. Tree hole crab water is administered to pregnant women, and particularly those with a history of miscarriages. The value of this water may relate to the behaviour of the crab, which neutralizes the naturally acidic water in tree holes by capturing snails and adding their crushed shells to the water, raising the pH and also enhancing levels of dissolved calcium (Bayliss 2002).

Likewise, the exoskeletons of *C. amnicola* (5.31%) and *C. armatum* (9.57%) are used in agriculture for soil fertilization. The reasons mentioned are related to the capacity of these shells (decomposed exoskeletons) to enrich the exhausted lands for better results. This attitude, based on unscientific experiences shows, that a part of the population of the study area has certain information on the stimulating power of substances contained in crab shells and their uses as biofertilizers. Kazemi and Salimi (2019) reported that chitosan, the linear polysaccharide obtained from crab exoskeletons, is good to improve productivity in agriculture. Chitosan is an effective organic molecule that improves productivity by supplying valuable nutrients to plant and due to site specific action of this nanoparticle it also enhances activity of protective enzymes that enables biotic and abiotic stress resistance in plants (Somdutt et al., 2019).

## Dynamics and management in the value chain of *C. amnicola* and *C. armatum*

The actors involved in the management and dynamics and value chain of crabs are fishermen/captors, wholesalers/collectors and retailers. The same observation was made by Sankaré et al. (2014a) who propose management measures for swimming crab *C. amnicola* stock in Aby-Tendo-Ehy lagoon complex in Ivory Coast. Products from fishing/capture are delivered to wholesalers/collectors who are at the second link in the chain. At this level of the chain, 40-50% of fishery products are exported to the markets of Togo and Ghana. The reasons reported for this attitude are related to financial profitability. Indeed, the external market is more profitable for the sellers. Both among the actors (fishermen/captors and retailers) involved in the distribution of products (*C. armatum* and *C. amnicola*) as well as among consumers, 100% of the exoskeletons resulting from post-capture losses as well as those that are derived treatments for consumption, are not used in any way. These poorly formed exoskeletons are, therefore, pollutants and a problem for the environment because there is no system in the study area for effective management of crab exoskeletons.

## Resource flow monitoring and socio-economic value of *C. amnicola* and *C. armatum*

Resource flow monitoring of *C. armatum* and *C. amnicola* shows the availability of both species throughout the year, but with higher levels of abundance between April and August. In the management of these resources, 40 to50% is exported to Togo and Ghana, likewise, post-capture as well as the exoskeletons of the crabs are not used in the study area. These exoskeletons can be used for the production of chitosan to improve crop yields. Chitosan is a biodegradable substance of natural origin obtained by the deacetylation of chitin, which is found in the exoskeletons of crustaceans such as lobsters, shrimps and crabs (Oanh et al., 2007).

The study also showed a very important socio-economic value of the crabs, *C. armatum* and *C. amnicola.* The actors involved in the crab value chain have incomes according to the species. Indeed, for *C. amnicola*, income range was from 8,000 ± 2,828 FCAF (retailers) to 15.500 ± 16.263 FCAF (wholesalers/ collectors). Besides, for *C. armatum*, income range was from 8750±1767 FCAF (retailers) to 20.000 ± 21.213 FCAF (wholesalers/collectors). Indeed, crabs management is a sector that provides significant income to the actors involved. Wholesalers/collectors are those who make more profit with higher added value 15.500 ± 16.263 FCAF in the sector. They are more or less organized and collaborate to maintain their power over the rest of the chain. The implementation of sustainable crab management strategies can significantly improve the income of actors involved in the crab sales chain.

## Conclusion

This study shows the availability of *C. amnicola* and *C. armatum* crabs in the study area. Besides, there is lack of means and system for managing the losses and exoskeletons of these crustaceans. In addition, the two species of crabs (*C. armatum* and *C. amnicola*) have a varied socio-cultural importance that resides in the various uses that populations make of them. These species are used for food (human and animal), medicinal and agricultural purposes. The exoskeletons of these crustaceans are not used in most cases. The actors involved in the value chain of the two species are fishermen/captors, wholesalers/collectors and retailers. The study found that crabs (*C. armatum* and *C. amnicola*) have remarkable socio-economic importance. Wholesalers/collectors are those who make more profit with high benefit in the sector, are more or less organized, and collaborate to maintain their power over the rest of the chain.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGMENTS

The authors are grateful to the community leaders (heads of districts, heads of villages), the survey guides, the actors involved in crabs' resources chain, persons and all others encountered during the survey for their openness and collaboration and to have shared their knowledge with us.

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Vol. 12(2), pp. 47-60, July-December 2020
DOI: 10.5897/IJFA2020.0783
Article Number: A9F0F1865584
ISSN 2006-9839

http://www.academicjournals.org/IJFA

International Journal of Fisheries and Aquaculture

*Full Length Research Paper*

# Water activity, microbial and sensory evaluation of smoked fish (*Mormyrus caschive* and *Oreochromis niloticus*) stored at Ambient Temperature, Terekeka-South Sudan

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Received 3 June, 2020; Accepted 11 September, 2020

**The effect of smoking kilns on water activity, microbial load and sensory attributes was evaluated to adopt suitable technology that maintains the quality of fish in South Sudan. A total of 300 fresh *Mormyrus caschive* and *Oreochromis niloticus* were purchased, 36 fresh samples were iced and the remaining 264 fish samples were divided into two batches for pit and *chorkor* smoking. The experiment was done twice in a completely randomized design. Fish samples were analyzed for water activity using Lab-Swift Meter, microbial load using standard microbiological methods and sensory characteristics using 9-point hedonic grading scale. Result revealed that, water activity in pit smoked fish increased at a rate of 1.7 times faster than in *chorko*r smoked samples during storage. Corresponding to water activity, plate count equally increased at a rate of 1.7 times faster for pit smoked *M. caschive* and 1.1 times for *O. niloticus* than samples smoked using *chorkor*. Similarly, mould load increased at a rate of 1.5 times faster for pit smoked *M. caschive* and 2.2 times for *O. niloticus* than samples smoked using *chorkor* oven. Overall, *chorkor* smoked fish had significantly better sensory parameters than pit smoked samples after one month's storage. Therefore, *chorkor* kiln produced quality smoked fish in terms of microbial load and organoleptic parameters, and the study recommends its adoption for artisan fisheries in South Sudan. However, microbial characteristics of smoked fish need to be further examined for a period exceeding a month to determine microbiological quality and establish fish self-life in order to maintain the quality and safety of fish and fisheries products.**

**Key words:** Water activity, microbial load, sensory attributes, smoking technologies, shelf life.

## INTRODUCTION

Fish is an important source of food and income to a large section of population in the developing world (FAO, 2015). In Africa, about 35 million people, depend wholly or partly on the fishing sector, mostly the small-scale

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fisheries, for their livelihoods (Adeyeye and Oyewole, 2016). In particular, fish is an important and cheap source of protein to low income earners in developing economies (Nunoo and Kombat, 2013). Fish forms more than 50% of the essential animal protein and mineral intake for over 400 million people from developing countries (FAO, 2016). In South Sudan, over 1.7 million people depend on fisheries for their livelihoods (FAO, 2018). The immense contribution and importance of artisan fishing to livelihoods in South Sudan increased from 6.8 to 10.2% from 2015 to 2016, indicating greater reliance on this source of income (FAO, 2018). In terms of employment and income source, FAO (2016) estimated that over ten billion people in the developing world are engaged in fisheries activities mainly as fishers, processors and vendors. However, fisherfolks mostly processors are faced with high post-harvest losses due to lack of proper processing and preservation techniques (Adeyeye et al., 2016; Amegovu et al., 2017). Poor handling of fish catches leads to significant physical, quality, nutritional and economic losses (Adeolu et al., 2017).

Equally, fish post-harvest handling provides livelihoods to millions of people and contributes significantly to foreign exchange of many countries in the world (FAO, 2016). Fish processing and preservation are important due to the fact that fish is highly susceptible to deterioration after harvest (Olopade et al., 2013; Getu and Misganaw, 2015), and to prevent quality, nutrient and economic losses (Golub, 2014). Hygienic handling of fish is essential to attain the best quality and highest possible profits (Muhame et al., 2020). Despite the fish vast importance, fishing industry suffers from enormous post-harvest losses which are estimated at 30% in dry season and 40% in rainy season (FAO, 2016). Fish post-harvest losses have a profound adverse impact on rural fishing population whose livelihoods often depend on fisheries activities (Adeyeye and Oyewole, 2016).

Lack of proper infrastructure for fish post-harvest handling is a major challenge facing rural fishing communities of South Sudan (Amegovu et al., 2017). As such, improved processing and preservation technologies are sought to reduce adverse effects of fish post-harvest losses. Among the traditional methods of salting, sun drying and fermentation, fish smoking is the main type of fish preservation technique used in Terekeka (UNIDO, 2015). This is due to the fact that most consumers prefer smoked fish to sun dried, wet salted and fermented fish in addition to technical simplicity (Magawata and Musa, 2015). Fish smoking is also commonly practiced in remote areas where delivery of fresh catches to distant markets is not permitted due to poor roads and lack of cool chain transport facilities (Adeyeye and Oyewole, 2016). The need to improve smoking technologies in such areas is of a paramount importance to reduce fish post-harvest losses (Emere and Dibal, 2013; Adeolu et al.,2017). Effective and efficient fish smoking indeed, maintain the quality and safety of fish due to antimicrobial, antioxidants and preservatives contained in the smoke (Omodara et al., 2016; Pemberton-Pigott et al., 2016; Yusuf and Hamid, 2017). The safety and quality of smoked fish help consumers in making decisions on processed fish products (Nunoo and Kombat, 2013), which also influence the market prices (Nguvava, 2013).

Fish smoking and drying reduce moisture, concentrates nutrients (Kumolu-Johnson et al., 2010), and reduce water activity in processed fish products (Pal et al., 2016). Well smoked fish with low water activity of less than 0.50 and moisture content between 15 and 25% is noted to inhibit growth of pathogenic microorganisms in the products (Francisca et al., 2010). During smoking process where salt is used, micro-organisms at the surface of fish are potentially inhibited (Oparaku and Mgbenka, 2012). Nyarko et al. (2011) reported mean microbial load in salted-smoked fish in the range of 4.79 to 6.52 log cfu/g which are within the acceptable limits for microorganisms in preserved foods as recommended by the International Standards. Olayemi et al. (2012) reported total bacterial and mould counts of $2.0\times10^4$ and $0.7\times10^4$ cfu/g, respectively, depending on fish species. Olayemi et al. (2012) added that microbial load is associated with the location of fish harvest, method of fishing, amount of salt used, smoking conditions, smoke chemicals and handling practices. Yusuf and Hamid (2017) noted that, contamination of dried fish by microorganisms occurs during processing, storage, transportation and during sale at open air markets.

Proper handling of fish during processing is noted to lower microbial count in the final products (Huong, 2014). However, the effect of smoking technologies on water activity, microbial load and sensory characteristics of smoked fish during storage at ambient temperature has not been determined in South Sudan. This study was therefore, conducted to assess water activity, microbial load and sensory attributes of pit and *chorkor* smoked *Mormyrus caschive* (L) and *Oreochromis niloticus* (L) stored at ambient temperature to enable fisherfolks to adopt suitable smoking technology that maintains quality and safety of smoked fish in South Sudan.

## MATERIALS AND METHODS

### The study area

The study was conducted in June, 2018 in Terekeka, South Sudan. Terekeka State is located at 70 km north of Juba on the western bank of the Nile (Miller and Benansio, 2011). Terekeka lies within Latitudes 5°26'38"N and Longitudes of 31°45'02"E, respectively (FAO and WFP, 2019). The state covers an area of about 10,358.95 km$^2$ and is occupied by an estimated population of more than 246,483 (South Sudan Centre for Census, Statistics and Evaluation, 2018). Terekeka has tropical climate with comparatively small seasonal variation of temperature, humidity and wind throughout the year. Terekeka usually receives rainfall between the months of April to November with an average annual rainfall of 907 mm. The area experiences dry periods between the months of December and March with an average annual temperature of

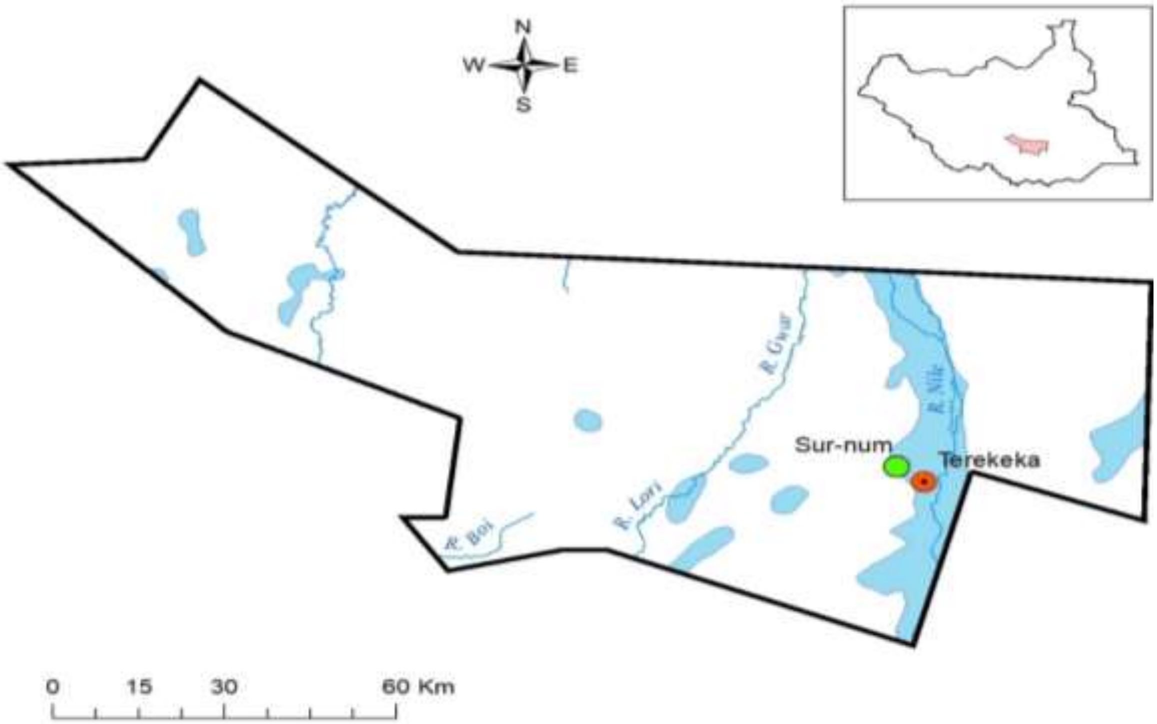



**Figure 1.** Map of the study area (Sur-num Landing Site, Terekeka State-South Sudan in Africa).

**Table 1.** Systematic positions, common and local names of the two fish species used for the study.

| Number | Family | Genus | Species | Common name |
|---|---|---|---|---|
| 1 | Mormyridae | Mormyrus | *Mormyrus caschive* | Elephant snout |
| 2 | Cichlidae | Oreochromis | *Oreochromis niloticus* | Nile Tilapia |

27.7°C. It is within the dry season that most people are actively involved in fisheries activities. However, plentiful fish catch occurs during the months of June to August particularly when the flood recedes (Figure 1).

**Study design**

The study was conducted in two phases, field experiment and laboratory analysis. Physical parameters of the two fish species were recorded in the field. Systematic positions according to Linnaeus (1758) and common names of the two fish species used for this study are shown in Table 1.

The lengths and weights were measured using meter rule and digital weighing scale, and reported as Means ± Standard Deviation. Experimental smoking using pit and *chorkor* smoking kilns was conducted twice in a completely randomized design. Women fish group *chorkor* oven was used to represent improved smoking technology. The *chorkor* measuring 2 m long, 1 m wide and 1 m high with 3 trays were attached after every 30 cm from base to top. The *chorkor* was constructed using unbaked bricks and the interior part plastered with clay soil. The top part roofed with perforated flat iron sheets. It has two inlets at the base for aeration and smoke production by burning firewood. The smoking chamber of *chorkor* oven has a movable door that remained closed except during monitoring.

Traditional pit was constructed alongside improved *chorkor* oven. Its dimensions were 1 m long, 0.5 m wide and 0.5 m high as practiced by fisherfolks in the area. Four wooden planks were placed at the edges where a wire mesh sits. During fish smoking, flat iron sheet was used to cover the samples. Fish smoked samples from the two kilns were wrapped in aluminium foils, labeled for easy identification, packed in containers and transported by vehicle to the laboratory in Makerere University, Uganda for analyses. While in the laboratory, samples were removed from the aluminium foil and kept at ambient temperature during the analysis to depict storage conditions provided by fish vendors in South Sudan. In the laboratory, three study parameters were determined to compare the two smoking technologies: water activity, microbial load and sensory characteristics (Figure 2).

**Sampling, processing and analysis procedures**

To cater for all the study parameters, a total of 300 fresh *M. caschive* and *O. niloticus* were purchased from fishermen at Sur-

**Figure 2**. The structures of Pit (left) and *Chorkor* (right) smoking ovens.
Source: Photograph taken by Charles Mondo on 26, June 2018.

num landing site situated at about 1 km from Terekeka town. Fish processing was done immediately after delivery. Fresh fish samples were kept in cooler boxes packed with ice blocks instantly after being harvested at the fishing grounds. Purposeful fish smoking using improved *chorkor* and traditional pit was conducted at Terekeka landing site. From the procured samples, 18 specimens of each species were ice stored at 4°C and transported to the laboratory where 6 specimens from each species were randomly picked and destined for microbial analysis. The remaining 282 specimens were divided equally into two batches, taking into account the species for pit and *chorkor* experimental smoking using *Acacia seyal,* the dominant tree species for smoking fish in the area.

All fish were washed to remove slime, descaled, eviscerated and rewashed thoroughly with clean water to remove blood. Prepared fish samples were immersed in freshly prepared salt solution (a mixture of 100 g salt in 10 L of clean water) for 15 min followed by draining for 15 min. Fire was set in pit and *chorkor* kilns to generate smoke heat by burning *A. seyal* wood. The pre-treated fish samples were randomly loaded on the wooden trays and wire mesh on top of *chorkor* and pit, respectively. The desired temperature of 60 to 80°C was maintained manually by a thermometer until the fish were smoke dried. During smoking, the position of fish samples in the wooden trays were changed in *chorkor* to attain uniformity of the products and turned upside down in pit kiln in mid periods in order to make samples smooth and steady in texture and appearance. Smoked fish were cooled for 12 h at ambient temperature. Smoked samples were wrapped in aluminium foils, labeled for easy identification, packed in containers and transported by road transport to the laboratory in Makerere University, Uganda for analyses. While in the laboratory, smoked fish samples were removed from the aluminium foil and kept at ambient temperature during the analysis period to depict the storage conditions provided by fish vendors in South Sudan.

**Determination of water activity in smoked fish samples**

For water activity, 10 g of fish muscles were aseptically removed from each fish species. The portion was placed in a round internal plate and inserted into the water activity meter (Lab Swift Bench Model Water Activity Meter, BS ISO 21807:2004; Public Health England, 2017). The meter set at 20°C, automatically read the water activity value in the sample after 5 min time.

**Determination of microbial load in fish samples**

Standard methods were used for identification and enumeration of microorganisms (ISO, 2003; 2008; ICMSF, 1986; BAM, 2005). Iron Agar and Dicloran Rose Bengal Chloramphenicol Agar used in this study were obtained from Oxoid Limited, England. These media were prepared and sterilized according to the manufacturer's instructions. Sterility control plates of each medium and diluent were made by incubating them overnight at a suitable temperature.

**Sample preparation for microbial load determination**

From the fish samples, a 10 g of each sample was removed from the muscle and aseptically weighed on an electronic balance. The weighed samples were blended in a stomacher 400 (Lab. Blender, London, UK) and transferred individually into 180 mL dilution bottles (Borosilicate-resistant glass with rubber stopper) containing 90 mL of sterile peptone water (0.1%) for suspension. This was done for samples of the two fish species obtained from each smoking technology and was taken as the original stock. The content was homogenized using vortex mixer and a dilution of 1:10 ($10^{-1}$) and 1:100 ($10^{-2}$) were obtained using standard serial dilution method (David and Davidson, 2014). The spread plate technique was used to culture microorganisms (Yusuf and Hamid, 2017). After incubation, the number of colonies on a dilution plate between 30 and 300 colonies were counted and the number of microorganisms was determined using ISO 4833:2003 (ISO, 2003) formula:

$$\mathrm{N} = \frac{\sum C}{V(n1 + 0.1n2)d}$$

where *N*= number of colonies per mL or gram of the sample, $\sum C$= sum of all colonies counted on the plates containing 30-300 colonies, *n1*= number of plates counted in the lower dilution, *n2*= number of plates counted in the higher dilution, *d*= value corresponding to the dilution from which the first counts were obtained and *V*= volume of inoculums used. The total viable count and mould count were recorded as colonies formed by microorganisms in the inoculated plates. The means of duplicate microbial count in colony forming units were determined and transformed to $\log_{10}$ cfu/g for statistical analyses. Media and air control plates were also prepared and carried out parallel to the analysis as internal quality control.

**Table 2.** Initial mean values of water activity and microbial load recorded in smoked *M. caschive* and *O. niloticus*.

| Parameter | *Mormyrus caschive* | | *Oreochromis niloticus* | |
|---|---|---|---|---|
| **Smoking types** | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| Water activity ($a_w$) | 0.62 | 0.53 | 0.62 | 0.56 |
| Total plate count ($log_{10}$ cfu/g) | 2.12 | 1.51 | 2.50 | 1.54 |
| Mould count ($log_{10}$ cfu/g) | 1.65 | 1.00 | 1.65 | 1.30 |

Values are means of duplicate determination of homogenized fish muscles.

#### Enumeration and identification of microorganisms

For total aerobic plate count, 1 mL of prepared dilution from each sample was spread on plates inoculated with Standard Iron Agar (IA) in duplicates. Plates were then aerobically incubated in an inverted position at 35 ± 2°C. Colonies were counted after 48 h of incubation using Reichert Dark Field Quebec Colony Counter. For moulds' isolation, a standard method of ISO 21527-1:2008 was used with minor modifications. Briefly, 1 mL of each dilution was spread on the surface of plate inoculated with Dicloran Rose Bengal Chloramphenicol Agar (DRBC Agar; Category 1160) using a sterile glass rod. DRBC Agar inoculated plates were incubated uprightly at 25 ± 1°C. Counting of mould colonies was done after 5 days of incubation using Quebec Colony Counter. Mould colonies were recorded as colony forming units per gram (cfu/g) of fish samples. The means of duplicate microbial count in colony forming units were calculated and transformed to $log_{10}$ cfu/g for statistical analyses.

#### Evaluation of organoleptic attributes of smoked fish

The organoleptic attributes: appearance, aroma, flavour, taste, texture, and the overall acceptability of smoked fish were evaluated. Preference and paired comparison test as recommended by FAO (1999) in the Codex guidelines for sensory evaluation of fish and shell fish in laboratories (CXG 31-1999) were used. The sensory attributes were assessed by a control test panel of 20 individuals using 9-point hedonic grading scale as described by Aba and Ifannyi (2013). Grading scales were described as "poor" for attributes assigned value (1 to 3), "fairly good" (4-5), "good" (6-7) and "very good" (8-9). Twenty staff members (control test panel) consisting of 10 males and 10 females of age range 25 to 45 years old from the National Agriculture Research Laboratories in Kawanda constituted the sensory evaluation panel. As a precaution, panelists were given 10 min break interval to wash their mouths using distilled water after every sample assessment to avoid carry over effect. Panelists were also prevented from communicating to each other during the assessment by allotting a booth to each member.

#### Preparation of smoked fish samples for sensory evaluation

Pit and *chorkor* smoked fish samples were differently cooked in a microwave container for 10 min at 121°C and allowed to cool to a comfortable tasting temperature. Then coded with 3 figures for example: 101, 102, 103 and 104 for pit and *chorkor* smoked *M. caschive* and *O. niloticus* assessed on day 0 (week one). Coded samples were randomly placed in a tray and a glass of clean water provided for panelists to cleanse their palate after each sample taste. Prepared fish samples were served to each booth where a participating panelist was assigned. Panelists indicated scores for preference and compare between samples obtained from pit and *chorkor* technologies. Assessors noted organoleptic tests and general acceptability of the four specimens and recorded their observation by assigning a value to each specimen.

#### Statistical analyses

Data collected from the study was analyzed using R statistical package (R Core Team, 2018). A two-way analysis of variance (ANOVA) was used to test the difference in water activity, microbial counts and sensory attributes recorded in fish samples smoked using *chorkor* and pit to determine the effect of smoking technologies on the quality parameters of fish. Tukey's Honest Significant Difference test was performed where the means of the two groups under comparison were significantly different. Pearson Correlation Analysis (PCA) was done to establish the relationship between the factors: water activity and microbial growth associated with sensory attributes of smoked fish. Statistical significance level was studied at $P \leq 0.05$.

## RESULTS

### Physical parameters of the two fish species

Average lengths and weights of the two fish species were 28.17±1.17 cm, 133.06±3.60 g and 23.31±1.01 cm, 126.73±1.86 g for *M. caschive* and *O. niloticus,* respectively.

### Water activity and microbial load in smoked *M. caschive* and *O. niloticus*

The initial mean water activity values in pit smoked fish were higher than values recorded in *chorkor* smoked *M. caschive* and *O. niloticus,* respectively (Table 2). Equally, the initial total plate count was significantly higher in pit than in *chorkor* smoked *M. caschive* and *O. niloticus,* respectively. Similarly, the initial mould count was significantly higher in pit than in *chorkor* smoked *M. caschive* and *O. niloticus,* respectively. Generally, *M. caschive* and *O. niloticus* smoked using *chorkor* oven had significantly lower microbial load than pit smoked samples ($P<0.05$).

### Water activity ($a_w$) determined in smoked fish during storage

Generally, water activity increased with increase in

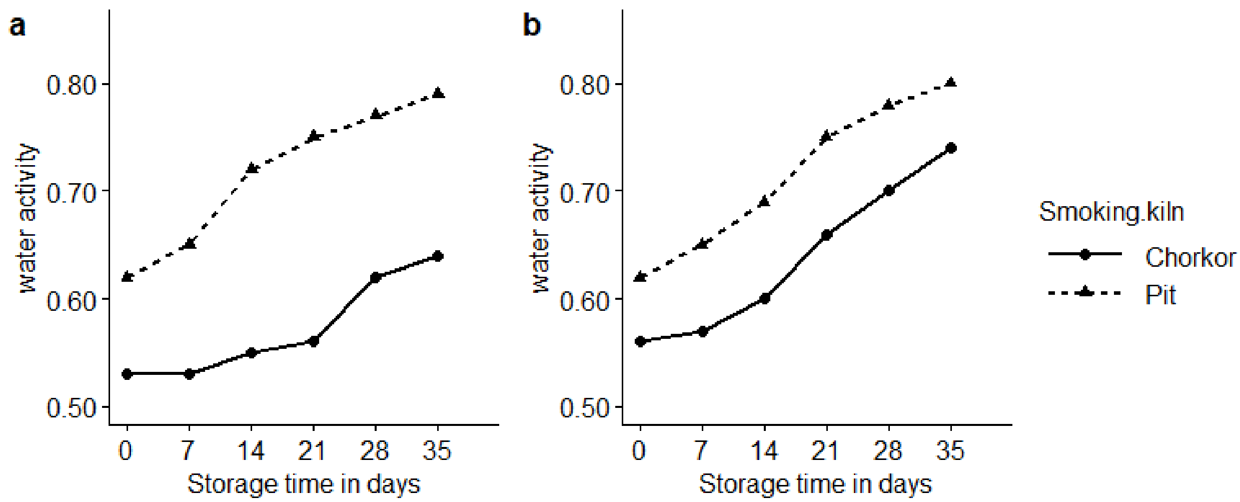



**Figure 3.** Trend in water activity recorded in pit and *chorkor* smoked *M. caschive* (a) and *O. niloticus* (b) stored for 35 days at room temperature.

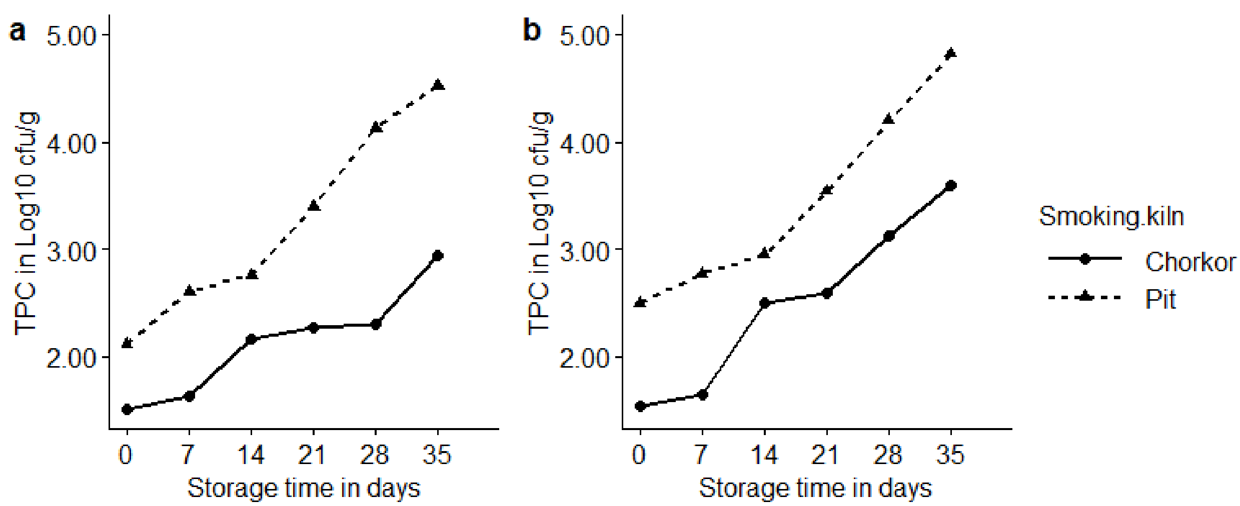



**Figure 4.** Trend in total plate count recorded in pit and *chorkor* smoked *M. caschive* (a) and *O. niloticus* (b) stored for 35 days at room temperature.

storage over the 35 days keeping time (Figure 3). The average rate of increase in water activity in pit smoked fish (0.005/week) was significantly higher than in *chorkor* smoked samples (0.003/week, P<0.05). With regards to smoking technologies, mean water activity recorded in pit smoked fish was significantly higher than that measured from *chorkor* specimens (P<0.05, Figure 3a and b).

## Total plate count of smoked fish during storage at ambient temperature

The study recorded an increasing trend in microbial load, total plate count (TPC) in the muscles of smoked fish during storage at room temperature (Figure 4a and b). Total plate count increased significantly with increase in storage time. With regards to the technologies, pit smoked *M. caschive* has significantly higher total plate count than *chorkor* smoked samples in the entire storage period (P<0.05). The growth rate of microorganisms in smoked fish products during storage revealed that, pit smoked *M. caschive* has higher average growth rate of total viable count (0.069 $\log_{10}$ cfu/g/week) than *chorkor* smoked fish samples (0.041 $\log_{10}$ cfu/g/week, P<0.05). Similarly, the total plate count of smoked *O. niloticus* increased with increase in storage time (Figure 4b). Pit

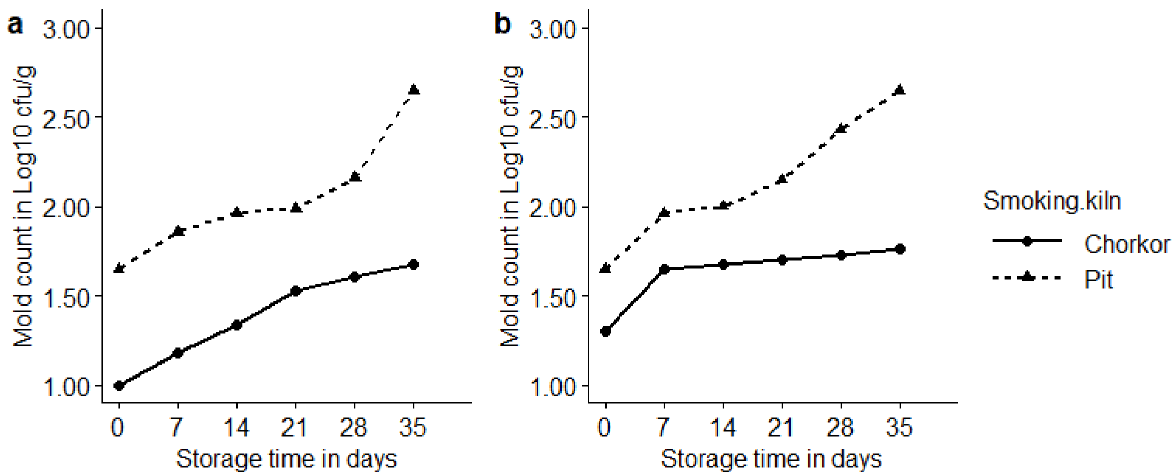



**Figure 5.** Trend in mould count recorded in pit and *chorkor* smoked *M. caschive* (a) and *O. niloticus* (b) stored for 35 days at room temperature.

smoked *O. niloticus* also has significantly higher total plate count than *chorkor* smoked samples (P<0.05). With regards to growth rate, pit smoked *O. niloticus* has higher average growth rate (0.066 $\log_{10}$ cfu/g/week) than *chorkor* smoked samples (0.059 $\log_{10}$ cfu/g/week). The increase in microbial load in smoked fish during storage strongly correlated ($r^2$=0.94) with increase in water activity. With regards to storage, the fourth and fifth weeks of storage showed higher water activity and microbial load than the first three weeks.

## Mould count of smoked fish during storage at ambient temperature

The study observed an increasing trend in mould count in the muscles of smoked fish during storage at room temperature (Figure 5). Moulds increased significantly with increase in keeping time (P<0.05). With regards to the technologies, pit smoked *M. caschive* has significantly higher mould count than *chorkor* smoked samples during the entire storage period (P<0.05). The average growth rate of mould was higher in pit smoked *M. caschive* (0.029 $\log_{10}$ cfu/g/week) than in *chorkor* smoked samples (0.019 $\log_{10}$ cfu/g/week). Similarly, smoked *O. niloticus* has significantly higher mould counts than *chorkor* smoked fish samples over storage time. The average growth rate of moulds was higher in pit smoked *O. niloticus* (0.029 $\log_{10}$ cfu/g/week) than in *chorkor* smoked samples (0.013 $\log_{10}$ cfu/g/week).

## Sensory evaluation of smoked *M. caschive* and *O. niloticus*

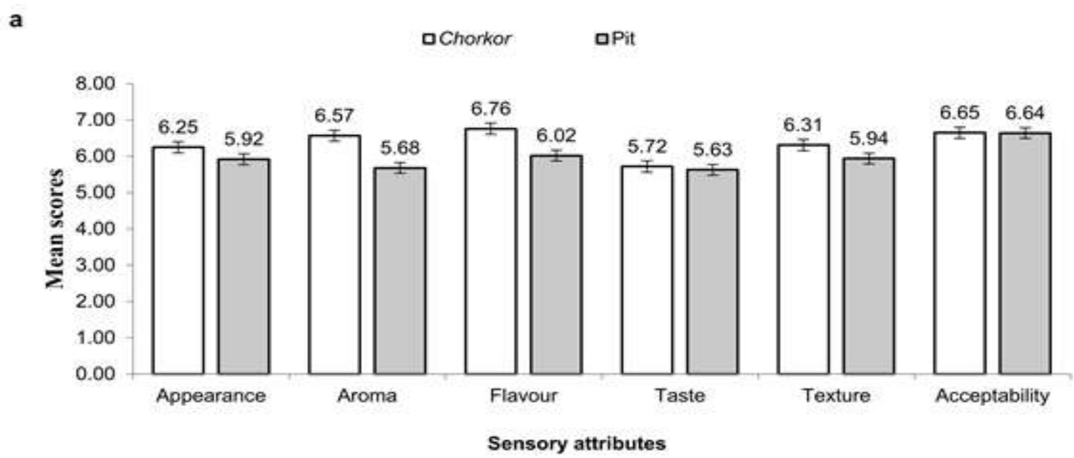
The mean score of organoleptic sensory tests of *chorkor* smoked samples were significantly higher than pit smoked *M. caschive* (P<0.05; Figure 6a). Meanwhile, the mean score for overall acceptability of fish smoked using both technologies did not significantly differ. With regards to *O. niloticus,* the mean scores of all the organoleptic sensory attributes of *chorkor* smoked fish were significantly higher than specimens smoked using pit kiln (P<0.05; Figure 6b). Comparison of means using Tukey's test showed a significant difference in the means of pit and *chorkor* smoked fish (P<0.05). All the organoleptic attributes have mean scores above 5, the critical limit for acceptability of smoked fish products under quality assessment.

With regards to the attributes, a decreasing trend was noted in the colour or appearance mean scores of smoked fish during storage (Table 3). The dark-brown colour of smoked *M. caschive* and *O. niloticus* decreased with increase in storage time. In terms of technologies, *chorkor* smoked *M. caschive* and *O. niloticus* had higher colour mean scores than pit smoked fish products. The difference was significant on the first week of the storage (P<0.05).

There was a decreasing trend in aroma of smoked fish with increase in storage time (Table 4). On the third and fourth week, the unique aroma of smoked fish decreased significantly with increase in storage time with *chorkor* smoked *M. caschive* and *O. niloticus* having better aroma than pit smoked fish (P<0.05).

The smoky flavour of pit and *chorkor* smoked *M. caschive* and *O. niloticus* decreased with increase in keeping time (Table 5). With regards to the technologies, *chorkor* smoked *M. caschive* and *O. niloticus* had better flavour than pit smoked fish products.

Similarly, the pleasant taste in smoked fish decreased with increase in storage time. Concerning the technologies, *chorkor* smoked fish had better mean taste



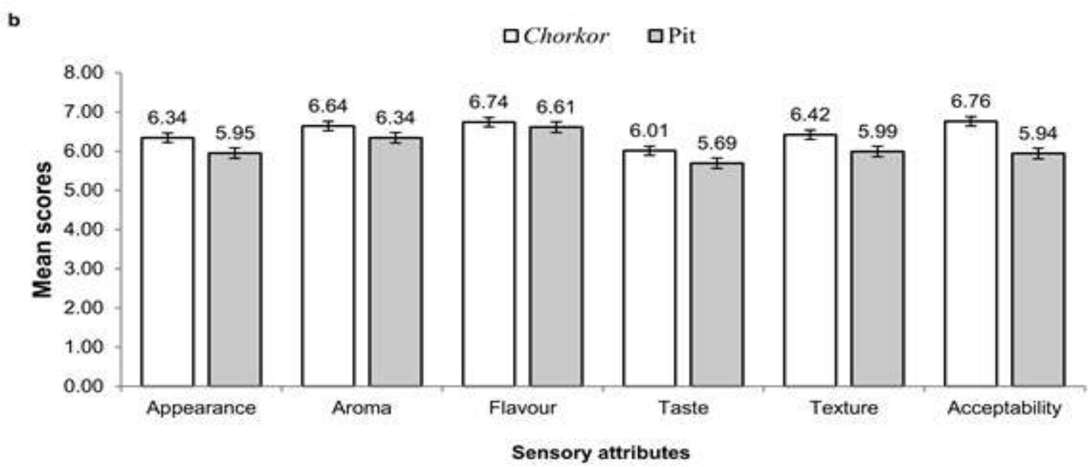




**Figure 6.** Overall sensory attributes of pit and *chorkor* smoked *M. caschive* (a) and *O. niloticus* (b) stored for 28 days at room temperature

**Table 3.** Trend in appearance or colour mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at room temperature.

| Parameter | Storage time (Days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | Pit | *Chorkor* | Pit | *Chorkor* |
| | 0 | $6.20 \pm 1.24^{b}$ | $6.50 \pm 1.67^{ab}$ | $6.60 \pm 1.98^{a}$ | $7.00 \pm 1.34^{a}$ |
| | 7 | $5.80 \pm 2.24^{b}$ | $5.80 \pm 2.04^{b}$ | $6.10 \pm 2.25^{b}$ | $6.25 \pm 1.41^{b}$ |
| Appearance | 14 | $5.75 \pm 1.80^{b}$ | $5.75 \pm 2.05^{b}$ | $5.65 \pm 1.90^{b}$ | $5.95 \pm 1.28^{b}$ |
| | 21 | $5.30 \pm 1.45^{c}$ | $5.35 \pm 1.18^{c}$ | $5.10 \pm 2.17^{c}$ | $5.65 \pm 1.23^{b}$ |
| | 28 | $5.10 \pm 2.17^{c}$ | $5.20 \pm 1.79^{c}$ | $5.00 \pm 1.75^{c}$ | $5.20 \pm 2.55^{c}$ |

Mean with different superscript in the same column for each parameter are significantly different (P<0.05). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

scores than pit smoked fish products (Table 6).

There were textural changes in pit and *chorkor* smoked fish. The mean textural scores in fish smoked using pit and *chorkor* technologies decreased with increase in storage time, and *chorkor* smoked fish had higher mean textural scores than pit smoked fish products (Table 7).

**Table 4.** Trend in aroma mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at room temperature.

| Parameter | Storage time (Days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| Aroma | 0 | $6.80 \pm 1.74^{a}$ | $6.80 \pm 1.77^{a}$ | $6.80 \pm 1.77^{a}$ | $7.20 \pm 1.61^{a}$ |
| | 7 | $6.05 \pm 1.93^{b}$ | $6.70 \pm 1.87^{a}$ | $6.70 \pm 1.87^{a}$ | $6.75 \pm 1.45^{a}$ |
| | 14 | $5.85 \pm 1.90^{b}$ | $6.65 \pm 1.27^{ab}$ | $6.65 \pm 1.27^{ab}$ | $6.70 \pm 2.07^{a}$ |
| | 21 | $5.25 \pm 1.45^{c}$ | $6.50 \pm 1.54^{ab}$ | $6.50 \pm 1.54^{ab}$ | $6.55 \pm 1.15^{ab}$ |
| | 28 | $5.00 \pm 1.35^{c}$ | $6.20 \pm 1.82^{b}$ | $6.20 \pm 1.82^{b}$ | $6.00 \pm 1.38^{b}$ |

Mean with different superscript in the same column for each parameter are significantly different ($P<0.05$). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

**Table 5.** Trend in flavour mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at ambient temperature.

| Parameter | Storage time (Days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| Flavour | 0 | $7.25 \pm 1.33^{b}$ | $7.50 \pm 1.43^{a}$ | $7.10 \pm 1.37^{b}$ | $7.25 \pm 1.37^{b}$ |
| | 7 | $6.55 \pm 1.82^{bc}$ | $6.90 \pm 1.59^{b}$ | $6.80 \pm 1.51^{b}$ | $6.90 \pm 0.85^{b}$ |
| | 14 | $6.10 \pm 1.33^{c}$ | $6.65 \pm 1.35^{b}$ | $6.50 \pm 1.79^{b}$ | $6.65 \pm 1.27^{bc}$ |
| | 21 | $5.15 \pm 1.60^{d}$ | $6.45 \pm 1.61^{c}$ | $6.40 \pm 1.70^{c}$ | $6.50 \pm 1.05^{bc}$ |
| | 28 | $5.05 \pm 1.54^{d}$ | $6.30 \pm 1.81^{c}$ | $6.25 \pm 1.80^{c}$ | $6.40 \pm 1.27^{c}$ |

Mean with different superscript in the same column for each parameter are significantly different ($P<0.05$). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

**Table 6.** Trend in taste mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at ambient temperature.

| Parameter | Storage time (Days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| Taste | 0 | $6.45 \pm 2.11^{b}$ | $6.90 \pm 1.29^{a}$ | $7.05 \pm 2.01^{a}$ | $7.25 \pm 1.59^{a}$ |
| | 7 | $6.25 \pm 1.59^{b}$ | $6.70 \pm 1.81^{a}$ | $6.50 \pm 1.88^{ab}$ | $6.55 \pm 1.47^{ab}$ |
| | 14 | $6.05 \pm 1.67^{b}$ | $6.50 \pm 1.64^{ab}$ | $5.80 \pm 2.28^{b}$ | $6.35 \pm 1.73^{b}$ |
| | 21 | $5.75 \pm 1.16^{c}$ | $5.85 \pm 1.95^{c}$ | $5.40 \pm 1.98^{d}$ | $6.00 \pm 1.52^{bc}$ |
| | 28 | $5.10 \pm 1.74^{d}$ | $5.30 \pm 2.00^{d}$ | $5.00 \pm 1.34^{d}$ | $5.55 \pm 1.96^{cd}$ |

Mean with different superscript in the same column for each parameter are significantly different ($P<0.05$). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

With regards to the overall acceptability, a decreasing trend was recorded in pit and *chorkor* smoked *M. caschive* and *O. niloticus* over storage time (Table 8). Low average acceptability scores were recorded on the last day than on the first day of the sensory evaluation. Concerning technologies, *chorkor* smoked fish were highly accepted than pit smoked fish. The overall acceptability means scores in pit and *chorkor* smoked *M. caschive* and *O. niloticus* were above 5, the critical limit for products under quality assessment.

Generally, evaluation of sensory attributes in pit and *chorkor* smoked *M. caschive* and *O. niloticus* during 28th days of storage depicted that smoked fish samples from *chorkor* technology were of better quality. Besides, *chorkor* smoked fish have higher mean scores in all the attributes than pit smoked fish products.

**Relationship between water activity and microbial growth with the sensory attributes of smoked fish stored at ambient temperature**

Pearson correlation results showed an inverse relationship between water activity and sensory characteristics with a range of -0.66 to -0.77 for the attributes. The lowest relationship was between water

**Table 7.** Trend in textural mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at ambient temperature.

| Parameter | Storage time (Days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| | 0 | 7.00 ± 1.56$^{a}$ | 7.35 ± 1.87$^{a}$ | 6.55 ± 2.01$^{ab}$ | 7.00 ± 1.45$^{a}$ |
| | 7 | 6.60 ± 1.19$^{a}$ | 6.65 ± 1.42$^{a}$ | 6.30 ± 1.56$^{b}$ | 6.70 ± 1.78$^{a}$ |
| Texture | 14 | 5.60 ± 1.85$^{bc}$ | 6.20 ± 1.58$^{b}$ | 6.10 ± 1.77$^{b}$ | 6.55 ± 2.26$^{ab}$ |
| | 21 | 5.45 ± 1.54$^{c}$ | 6.05 ± 1.28$^{b}$ | 5.55 ± 2.04$^{bc}$ | 6.35 ± 1.63$^{b}$ |
| | 28 | 5.05 ± 1.88$^{c}$ | 5.30 ± 1.72$^{c}$ | 5.45 ± 1.99$^{c}$ | 5.50 ± 1.47$^{bc}$ |

Mean with different superscript in the same column for each parameter are significantly different (P<0.05). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

**Table 8.** Trend in acceptability mean scores recorded in Pit and *Chorkor* smoked fish products stored for 28 days at ambient temperature.

| Parameter | Storage time (days) | *M. caschive* | | *O. niloticus* | |
|---|---|---|---|---|---|
| | | **Pit** | ***Chorkor*** | **Pit** | ***Chorkor*** |
| | 0 | 6.80 ± 1.64$^{a}$ | 7.15 ± 1.46$^{a}$ | 7.05 ± 1.36$_{a}$ | 7.25 ± 0.97$^{a}$ |
| | 7 | 6.40 ± 1.54$^{b}$ | 7.00 ± 1.75$^{a}$ | 6.80 ± 1.54$^{a}$ | 6.95 ± 1.50$^{a}$ |
| Acceptability | 14 | 6.20 ± 1.36$^{b}$ | 6.85 ± 1.31$^{a}$ | 6.65 ± 1.76$^{ab}$ | 6.70 ± 1.45$^{a}$ |
| | 21 | 5.35 ± 1.04$^{c}$ | 6.50 ± 1.54$^{ab}$ | 6.45 ± 1.54$^{b}$ | 6.60 ± 1.19$^{ab}$ |
| | 28 | 5.15 ± 1.69$^{c}$ | 5.75 ± 1.80$^{b}$ | 6.25 ± 1.02$^{b}$ | 6.30 ± 1.42$^{b}$ |

Mean with different superscript in the same column for each parameter are significantly different (P<0.05). SD=Standard deviation, Means ±SD are values of duplicate evaluation.

activity and appearance (r = -0.68), and highest between water activity and taste (r = -0.77). As water activity increases, the sensory quality of smoked fish reduces. Similarly, an indirect relationship between microbial load and sensory characteristics with a range of -0.70 to -0.81 was established for the attributes. The lowest relationship was between microbial growth and aroma (r = -0.70), and highest between microbial growth and taste (r = -81). As microbial load increases the sensory characteristics of smoked fish reduces, a reason for reduced acceptability of smoked fish during storage. Conversely, a direct and positive relationship between water activity and microbial load was established. As water activity increases, microbial load in smoked fish increases during storage with correlation value of 0.95. Equally, a positive and direct relationship between sensory characteristics of smoked fish stored at room temperature was established with a correlation range of 0.68 to 0.94 for the sensory attributes. The lowest relationship was between appearance and aroma (r = 0.68), and highest between appearance and taste (r = 0.94), and aroma and acceptability (r = 0.94).

## DISCUSSION

### Water activity in the muscles of smoked *M. caschive* and *O. niloticus*

Results of this study revealed that, the initial water activity in pit smoked fish was higher than recorded in *chorkor* smoked products. This is attributed to inefficient smoking that could not effectively remove water from fish tissues due to difficulties in controlling smoke heat and oxygen during the smoking process, given the openness of pit kiln. During storage, water activity increased with increase in storage time due to exposure of smoked fish to atmospheric air in the storage room. The rate of increase was higher in pit than in *chorkor* smoked fish samples due to higher initial water activity recorded in pit smoked fish (Ayeloja et al., 2018). Higher water activity allows microorganisms to infest the cracks, surfaces and the cavities or openings that subsequently digest smoked fish tissues.

### Microbial load in the muscles of smoked *M. caschive* and *O. niloticus*

Consistent with Udochukwu et al. (2016) and Yusuf et al. (2017), the study recorded higher microbial count in fresh fish than in smoked fish samples. This could be attributed to unhygienic handling of fish at the landing site and during transportation (Amegovu et al., 2017; Baniga et al., 2017; Muhame et al., 2020). It could also be due to the fact that, the ice used for preserving the fresh fish melted during transportation owing to temperature differences. Indeed, microorganisms increased exponentially with increase in ambient temperature

(Moneim et al., 2012; Pal et al., 2016). Concerning smoking technologies, *chorkor* smoked fish recorded lower microbial count than pit smoked fish. This agrees with earlier studies (Nunoo and Kombat, 2013; Agu et al., 2013; Adeyeye et al., 2015). Low microbial load could be attributed to proper handling of fresh fish for smoking and sanitary *chorkor* facility (Osvaldo, 2016), effect of smoke chemicals and heat in preventing or reducing the proliferation capacity of microorganisms (Adeyeye et al., 2015). In pit kiln, fish smoking is done in an open and unhygienic environment, hence the chances for microbial contamination (Adelaja et al., 2013; Adeyemi et al., 2013; Omodara et al., 2016; Amegovu et al., 2017).

In relation to storage, results revealed an increasing microbial load in smoked fish with storage time. This observation is in line with the findings of Kumolu-Johnson et al. (2010) who reported an increase in microbial load in smoked *Clarias gariepinus* during storage and attributed it to storage temperature and duration. Ayinsa and Maalekuu (2013), Ayuba et al. (2013) and Olatunde et al. (2013) reported that the quality of smoked fish deteriorates with time during storage at ambient temperature. The decline was attributed to increase in microbial load associated with increase in moisture content absorbed from the atmosphere (Daniel et al., 2013; Udochukwu et al., 2016). Relative increase in microbial load of smoked fish stored at room temperature is an important indicator of quality, and influences the time in which smoked fish can be stored (Kumolu-Johnson et al., 2010; Adeyemi et al., 2013). In the present study, increase in water activity due to moisture reabsorption during storage corresponded with increase in microbial load. This supports the observation that microbial load correlates well with water activity and storage time (Ayuba et al., 2013). Indeed, dehydrated fish easily absorbs water from the surrounding air into their tissues triggering microbial growth with subsequent increase in the load (Daniel et al., 2013). Despite the increase of microbes in smoked fish, the loads were below the maximum permissible limits recommended by the International Standards ($1.0\times10^6$ and $1.0\times10^4$ cfu/g) for bacteria and fungi, respectively.

Similarly, the study recorded higher mould count in fish smoked using pit than *chorkor*, and the load increased during storage at ambient temperature. This could be due to poor processing techniques employed in traditional pit kiln (Sajib et al., 2015) and exposure of the products during storage (Udochukwu et al., 2016). In addition, the findings showed moulds are capable of growing at water activity of 0.50. This is in line with Daniel et al. (2013) who revealed that moulds are the major microorganisms thriving in smoked and dried fish products with low water activity (0.60). Concerning microbial load and storage, it is imperative to say that pit smoked fish has low shelf life than *chorkor* smoked fish and consumption of such fish after 35th day of storage may result to potential health effects (Udochukwu et al., 2016).

**Sensory evaluation of organoleptic attributes of smoked fish products**

The overall results for sensory evaluation (Figure 5a and b) showed higher scores in *chorkor* smoked *M. caschive* and *O. niloticus* than pit smoked fish. With regards to the technologies, *chorkor* products had higher colour mean scores than pit smoked samples. Unlike the black and unattractive colour observed from pit smoked fish, *chorkor* products had attractive dark-brown colour. This could be due to the difference in proximity of fish to heat source, deposition and adsorption rate of smoke chemicals by fish surface during smoking. The colour difference could also be due to variation in smoking parameters that is, time and temperature as fish samples from pit were directly exposed to intense smoke heat for a longer period (18-24 h) than products from *chorkor* kiln (6-12 h). Besides, the difference could be due to the effect of oxidation or rancidity that might have occurred during smoking and storage. Studies observed that large amount of polyunsaturated fatty acids found in fish lipids make smoked fish liable to oxidation and autocatalytic reactions which may lead to production of higher peroxides during storage (Nguvava, 2013; Olukayode and Paulina, 2017).

In relation to taste, *chorkor* smoked *M. caschive* and *O. niloticus* have better taste scores than pit smoked fish. This could be due to salting effect and regulated smoking events which might have prevented fish products from contamination with microorganisms, external materials and smoke chemicals (Likongwe et al., 2018). Generally, the taste of smoked fish deteriorated during storage at ambient temperature with higher rate of taste decline recorded in pit smoked fish than in *chorkor* smoked products. This could be attributed to variation in smoke concentration which could have resulted to fish contamination by organic pollutants (Dutta et al., 2018), and difference in salt absorption from the surface into fish muscles. Besides, increase in water activity increases microbial load, and this reduces the taste of smoked fish during storage (Adeyeye et al., 2015). The rate of taste reduction differed between pit and *chorkor* smoked fish which is depending on the amount of water activity that directly influences microbial growth (Pal et al., 2016).

With regards to aroma, *chorkor* smoked fish had higher aroma mean scores than pit smoked products but a general decline in the pleasant aroma of fish was noted during storage. This difference could probably be due to variation in the rate of fat and protein decomposition due to heat treatment and subsequently, oxidation of poly-unsaturated fatty acids (PUFA) contained in fish muscles to products such as peroxides, aldehydes, ketones and free fatty acids during storage at ambient temperature (Olatunde et al., 2013; Stratev et al., 2015; Abdul-Baten et al., 2020). The difference in aroma could also be attributed to decomposition of nutrients by microorganisms during storage (Adeyemi et al., 2013;

Famurewa et al., 2017). This is evidenced by the inverse relationship between water activity and microbial growth with aroma. As water activity increases, microbial load also increases that reduced the sensory characteristics of smoked fish particularly aroma.

For flavour, *chorkor* smoked fish had better mean scores than pit smoked fish but a general decline in score was recorded in all smoked fish products during storage. Pre-treatment of fish with salt prior to smoking could have caused the difference as salt contributes to development of aroma and flavour (Odoli, 2015). Reduction in flavour during storage could probably be due to absorption of water owing to difference in osmotic pressure which existed between salted smoked fish and humidity. Moisture adsorbed increases water activity which directly influenced microbial growth (Pal et al., 2016). Due to increase in water activity, microorganisms are enabled to decompose proteins and fats to products such as amines, ammonia, peroxides and free fatty acids that may result to off flavour and rancid taste. This supports Famurewa et al. (2017) observation that fish nutrients are metabolized and reduced as spoilage parameters increases during storage.

In terms of texture, fish products smoked in *chorkor* were highly preferred by the panelists to pit smoked samples. This could be due to low water activity, and application of salt that retarded the activities of spoiling microorganisms and other chemical reactions (Reza et al., 2015), which would have contributed to undesirable textural changes. In line with other studies (Saritha et al., 2012; Adeyeye et al., 2015; Jakhar et al., 2015), variation in the overall acceptability of smoked fish could be attributed to efficacy of the technology in removing water that has an impact on visual and nutritive quality of smoked fish (Abdul-Baten et al., 2020).

The overall acceptability was higher for fish smoked using *chorkor* than pit. This is because the temperature used for smoking fish was not excessively high (60-80°C) as it was reasonably controlled. This has made most of the physical and nutritive qualities to be retained at the end of the smoking process, giving rise to products which were very pleasant, delicious and attractive as judged by the panelists. During storage, an inverse relationship between water activity and microbial growth with acceptability of smoked fish was established. As water activity increases so is microbial load that reduced the acceptability of smoked fish (Dutta et al., 2018).

On average, all fish smoked using pit and *chorkor* technologies were acceptable 28th days after smoking. This could be due to curing of fresh fish with salt prior to smoking that improves the taste, palatability and flavour (Magawata and Musa, 2015; Odoli, 2015; Ginigaddarage et al., 2018). Most importantly, salting of fish before smoking reduces and controls the activities of microorganisms (Chakraborty and Chakraborty, 2017) which may otherwise metabolize proteins, fats and poly-unsaturated fatty acids (PUFA) in fish muscle to oxidation products such as amines, ammonia, peroxides, aldehydes, ketones and free fatty acids during storage at ambient temperature (Ayeloja et al., 2018) which may cause changes in colour, aroma, flavour and texture of fish (Olatunde et al., 2013).

Most important to note is that, in all the sensory attributes examined, *chorkor* smoked fish scored higher than pit. However, all smoked fish scored above average, which indicates that pit and *chorkor* smoked fish might still be appealing and acceptable beyond a period of one month after smoking. However, the microbial load needs to be taken into account. In agreement with earlier studies (Daniel et al., 2013; Odoli, 2015; Ginigaddarage et al., 2018), fish smoked using *chorkor* might have longer shelf life than fish smoked using pit due to low water activity, low microbial load, and good sensory characteristics as judged by the panelists.

## Conclusion

The study revealed that *chorkor* removes water in fish more significantly than pit. It is also associated with low microbial load and good quality sensory parameters. Regardless of smoking technology, fish tissues continuously absorb atmospheric moisture during ambient storage resulting in increased water activity that led to increase in microbial loads. Despite rise in microbial load, fish were safe for human consumption as the loads were below the maximum permissible limits specified by the International Standards. The study recommends adoption of improved *chorkor* characterized with easy control of smoking parameters and maintenance of smoked fish quality. Uptake of improved *chorkor* will consolidate smoked fish value chain by maintaining the quality and quantity of smoked fish products that will enhance the role of fish in providing food and nutrition security in South Sudan, Africa.

## CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

## ACKNOWLEDGEMENTS

The authors acknowledged the editors, the anonymous reviewers whose comments have greatly enriched and improved this manuscript. This study was supported financially by Sudd Ecology and Management Project (NORHED Project Number SSD 13-0021). The Department of Environmental Management, College of Agricultural and Environmental Sciences, Makerere University is thanked for hosting this research. Thanks are also extended to Akullo Davies and Tinyori Edgar Samuel for technical support and cooperation.

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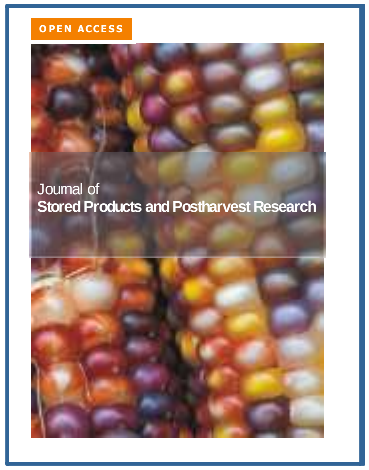


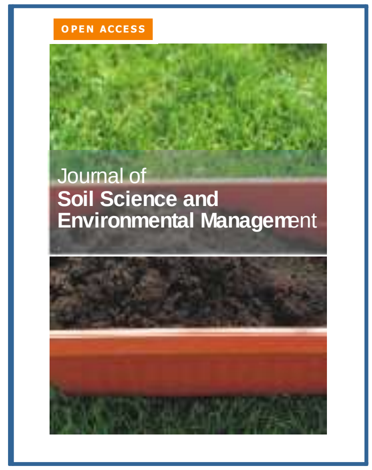




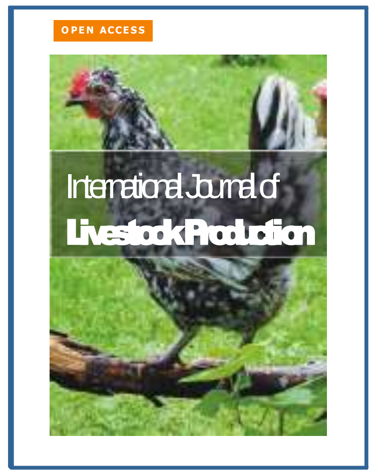




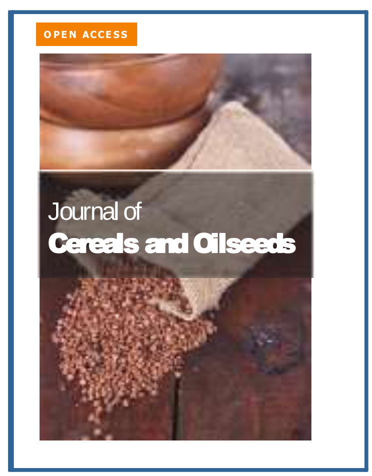


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